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THE INCIDENCE OF DRUGS IN FATALLY INJURED DRIVERS

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16. Abstract

Method for the collection of blood, urine, bile and alcohol washes of face and fingers from fatally injured drivers have been developed. Specimens have been collected from Alcohol Safety Action Project areas and other cooperating areas. The samples were supplied by coroners and medical examiners from fatally injured drivers who were dead on arrival at the hospitals. Nine hundred and twenty-nine specimen collection kits were distributed to 44 different areas. One hundred and ninety-one kits were returned with specimens from 18 areas. Methods for analysis of blood, urine and bile for 44 commonly abused drugs were developed. These methods consisted of extraction of the fluids, followed by a qualitative thin-layer chromatographic screen. If the screen indicated positives, quantitative confirmation was conducted. Mass spectrometry was also conducted if additional qualitative information was necessary. Alcohol washes of face and fingers were examined for evidence of marihuana using a thin-layer chromatographic method. Blood samples were assayed for alcohol content using a gas chromatographic method. The analytical results indicated that 51% of the drivers had ingested alcohol and 33% of the drivers were legally drunk (alcohol content of blood $\geq$ 0.15%). Twenty-four percent of the specimens examined evidenced the presence of drugs other than alcohol: 11% evidenced drugs and no alcohol; 13% evidenced drugs and alcohol.

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PREFACE

This report contains the accomplishments and results of a 15-month program designed to determine the incidence of drugs in fatally injured drivers. The report covers work conducted during the period 18 June 1971 to 18 September 1972. The project leader is Dr. E. J. Woodhouse, Senior Chemist, assisted by Mr. R. A. Adams, Associate Chemist, Miss J. Huerner, Assistant Chemist and Mrs. S. Reich, Assistant Chemist.

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SUMMARY

Methods for the collection of blood, urine, bile and alcohol washes of face and fingers from fatally injured drivers have been developed. Specimens have been collected from Alcohol Safety Action Project areas and other cooperating areas. The samples were supplied by coroners and medical examiners from fatally injured drivers who were dead on arrival at the hospitals. Nine hundred and twenty-nine specimen collection kits were distributed to 44 different areas. One hundred and ninety-one kits were returned with specimens from 18 areas. Methods for analysis of blood, urine and bile for 44 commonly abused drugs were developed. These methods consisted of extraction of the fluids, followed by a qualitative thin-layer chromatographic screen. If the screen indicated positives, quantitative confirmation was conducted. Mass spectrometry was also conducted if additional qualitative information was necessary. Alcohol washes of face and fingers were examined for evidence of marihuana using a thin-layer chromatographic method. Blood samples were assayed for alcohol content using a gas chromatographic method. The analytical results indicated that 51% of the drivers had ingested alcohol and 33% of the drivers were legally drunk (alcohol content of blood ≥ 0.15%). Twenty-four percent of the specimens examined evidenced the presence of drugs other than alcohol: 11% evidenced drugs and no alcohol; 13% evidenced drugs and alcohol. No specimens indicated any presence of marihuana.

I. INTRODUCTION

This report, the final report in a series of 14 reports, details the accomplishments, results and conclusions of a 15-month project designed to determine the incidence of drugs in fatally injured drivers. Specific objectives of the project were to develop methods for acquisition and drug analysis of specimens from up to 500 fatally injured drivers. The project involved development of kits for acquisition of specimens, development of analytical methods for screening specimens for drugs, acquisition of specimens and analysis of specimens.

Described below are the research approach and methodology, experimental procedures, experimental results, analysis and interpretation of experimental results, conclusions and recommendations and finally, project participants.

II. RESEARCH APPROACH AND METHODOLOGY

The National Highway Traffic Safety Administration is seeking a determination of the significance of drugs in highway fatalities. In order to accomplish this, a comparison must be made between the incidence of drugs in highway fatalities and the incidence of drugs in living drivers. The project described in this report was designed to investigate the incidence of drugs in highway fatalities--specifically the incidence of drugs in fatally injured drivers.

The research approach and methodology of this project are described below. (Specific details of operation are to be found in Section III - "Experimental Procedures.")

In order to gain useful information from which a determination of the incidence of drugs in fatally injured drivers could be made, the following research plan was adopted:

Midwest Research Institute (MRI) would assemble and distribute specimen-collecting kits containing equipment, instructions and identification (ID) cards to ASAP areas and other areas for the collection of specimens.

The ASAP directors and others would distribute the kits to coroners and medical examiners who were in a position to obtain specimens.

The coroners and medical examiners would return the specimens to MRI complete with an ID card. An identical ID card would also be sent by the coroners or medical examiners to the ASAP or area director.

MRI would, in the meantime, develop analytical methods for screening the specimens for drugs. The methods would be qualitative and quantitative. Forty-four commonly used drugs, cannabinoids, and blood alcohol would be screened for.

Upon receiving specimens, MRI would analyze them for the drugs in the screen. The analytical results would be forwarded to both DOT and the ASAP directors, coroners, or medical examiners from whom the specimens originated.

Finally, DoT would issue "Request for Crash Data" forms which MRI would distribute to all areas from which specimens have been received. These forms would then be returned to MRI complete with all pertinent information about the crash involved.

The information resulting from this program would then be subjected to evaluation and analysis to yield a determination of the incidence of drugs in fatally injured drivers.

Figure 1 illustrates the major activities, information and materials flow for the total program. Since this was the first program of its type, a major effort was expended in development of methods both for acquiring specimens and analysis of specimens. Certain decisions had to be made concerning the specimens, drugs and analyses, and after due consultation with NHTSA and experts in the field, the following important decisions were made:

To acquire specimens from fatally injured drivers who were dead on arrival at the hospital.

To acquire blood, bile and urine specimens, if possible.

To acquire face and finger washings.

To analyze blood, bile and urine for 44 more commonly abused drugs. These analyses were to be both qualitative and quantitative.

To analyze blood samples quantitatively for alcohol.

To analyze face and finger washings qualitatively for cannabinoids (marihuana).

This research plan involved the coordination of many persons and agencies. The success of the plan depended on the cooperation of all concerned, including DoT, MRI, ASAP area directors, coroners, medical examiners and other potential sources of samples. The accomplishments of the program are detailed in the next section of this report, "Experimental Procedures."

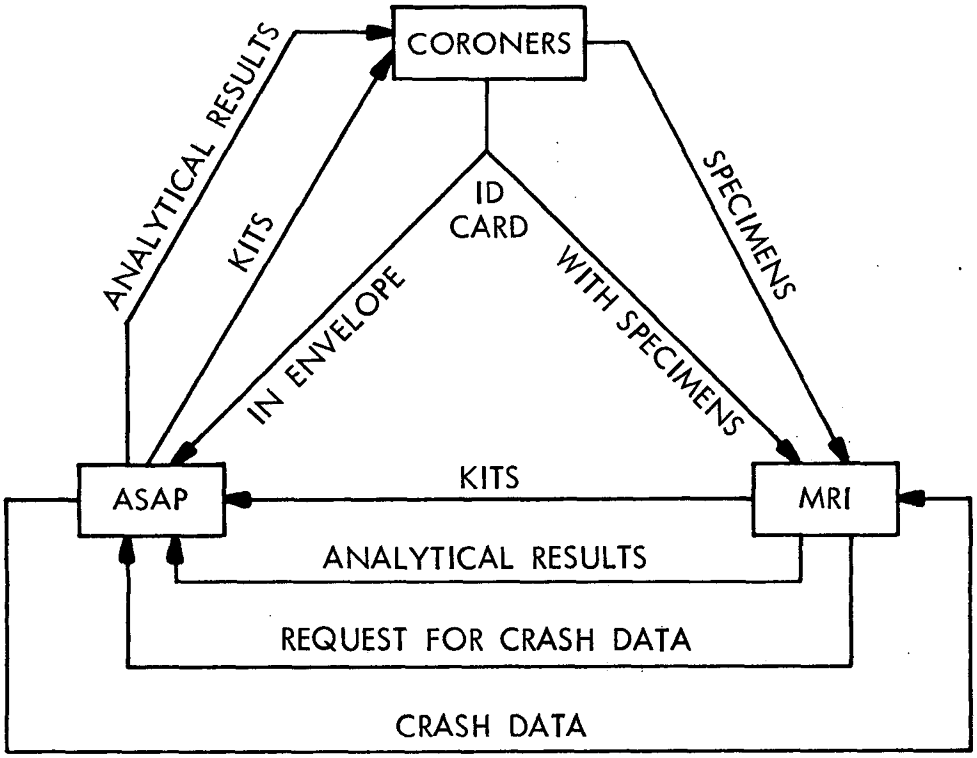

Figure 1 - Diagrammatic Representation of Information and Materials Flow for the Acquisition of Data on Fatally Injured Drivers.

III. EXPERIMENTAL PROCEDURES

This section details accomplishments in the various phases of the program. The following operations are described in order:

A. Preparation of Specimen Collection Kits

B. Acquisition of Specimens

C. Development of Analytical Procedures

D. Analysis of Specimens

E. Dissemination of Analytical Information

A. Preparation of Specimen Collection Kits

The specimen collection kits for this program were designed for the collection of urine, blood, bile, and face and finger washings. All the equipment for collection of specimens was included in the kit, which also contained full instructions and two identification cards, one for return to MRI and one for mailing to the local ASAP director for his files and future reference.

A specimen kit specifically consists of the following items:

1. A fully telescopic card box, 6-1/2 in. x 3 in. x 3 in., with MRI return address and air mail postage paid.

2. A urine collection bottle, 50-ml size, with superior quality screw cap seal, totally constructed of shatter-proof polypropylene, labeled "URINE".

3. A bile collection bottle, 30-ml size, similar to the urine bottle, labeled "BILE," and containing preservative (fluoride).

4. A blood collection kit consisting of a plastic bag containing a vacutainer holder, a vacutainer blood collection needle, and three 15-ml vacutainers treated with anticoagulant (oxalate) and preservative (fluoride).

5. A marihuana face and finger wash kit consisting of a plastic bag containing three enclosed, protected swabs labeled "right hand," "left hand," and "mouth;" and a small vial containing ethanol.

6. An instruction sheet detailing (a) requirements, and (b) how to use the kit.

7. An identification card in duplicate, enclosed in a protective plastic bag.

8. An envelope addressed to the local ASAP director for use in returning one of the identification cards.

These kits were assembled and dispatched to ASAP directors and others upon request. As the program proceeded, records were kept of the kit disposition for each area in order that each area could be constantly supplied with enough kits. At least 100 kits, fully assembled, were kept on hand at MRI at all times.

Figure A1 indicates typical components of a specimen kit. Figures A2 and A3 indicate the instruction sheet and the identification (ID) card, respectively. These figures are located in Appendix A "Acquisition of Specimens."

B. Acquisition of Specimens

Alcohol Safety Action Program (ASAP) directors, coroners, and medical examiners in 44 areas have been contacted by both DoT and MRI in an effort to acquire specimens from fatally injured drivers. Letters of program explanation, program requirements, requests for samples and memoranda were sent to all areas. Trial kits with full instructions were also dispatched to these areas. The response from 29 areas was sufficiently encouraging that these areas were supplied with sufficient collection and mailing kits to initiate specimen collection and mailing to MRI for analysis. Of these 29 areas, 18 have responded as of August 31, 1972, with a total of 191 specimen sets. Table A1 indicates the total areas contacted, specimen kits dispatched and specimen kits received. As of August 31, 1972, MRI has dispatched a total of 929 specimen collection kits and received 191 back. Table I indicates the number of specimen kits received per month during the project, and it can be seen that the number is steadily increasing. Many areas are considering cooperation, and 13 areas have only been contacted within the last 4 months of the program.

Our rapport with the supply areas is good; much of this is due to the efforts of Dr. J. L. Nichols of the Office of Alcohol Countermeasures, who has been in contact with all the potential areas we have requested cooperation from.

TABLE I

RECEIPT OF SPECIMEN KITS BY MONTH

Month	Number of Specimen Kits Received
June to November 1971	0
December 1971	8
January 1972	12
February 1972	6
March 1972	13
April 1972	23
May 1972	25
June 1972	33
July 1972	32
August 1972	39
Total	191

A complete listing, area by area, of the correspondence we have had with each area accompanies this report as a separate document. This listing was compiled as the program progressed, and include copies of all letters, memoranda, etc., sent to each area and the status of each area as of August 31, 1972.

The condition of most specimen kits was good when they were returned to the Institute. Although we had requested samples of urine, blood, bile and alcohol washings in each case, it was not always possible for the coroners or medical examiners to furnish all of these items. Table AII lists the specimen kits received up until August 31, 1972, their origin, and the status of the contents. Out of the total 191 specimen kits, 100% furnished the alcohol washes, 63% furnished urine, blood and bile, 81% furnished urine, 97% furnished blood, and 80% furnished bile.

At the initiation of this project, we also requested that liver samples be included in the program. The response from coroners and medical examiners persuaded us that we were likely to get much better cooperation from them if we requested only urine, blood, bile and alcohol washings. Liver samples were, therefore, dropped from the request before any kits were dispatched. Liver is a good source for analysis of drugs, but since we were already requesting urine, blood, and bile, we felt that if drugs had been taken, we would find them in at least one of the fluids requested.

C. <u>Development of Analytical Procedures</u>

The analytical procedures developed for this program consisted of a qualitative thin-layer chromatography (TLC) screen followed by a quantitative gas chromatographic (GC) confirmation of positives from the TLC screen. If any doubt existed as to the nature of a drug, mass spectrometric analysis was also conducted (qualitative). TLC and GC methods were initially chosen for their already proven reliability to detect and quantitate many drugs in body fluids.

The above tests were carried out on extracts from the body fluids or alcohol washes. Various extraction systems were investigated for their suitability with the above analytical methods.

In order to quantitate drug levels in body fluids, the extraction efficiency of the system was also determined for the drugs which were confirmed present in body fluids during the course of this program.

Blood alcohol levels were also determined in this project using a gas chromatographic technique. Described in detail below are the various stages of the methods development program. They are:

1. Investigation of the characteristics of pure samples of the drugs to be screened for.

2. Investigation of extraction systems on body fluid solutions of the drugs to be screened for.

3. Determination of extraction efficiencies.

4. Investigation of hydrolysis of specimens.

5. Development of analytical methods for marihuana.

6. Development of a total analytical system for the drugs to be screened for.

7. Determination of blood alcohol levels.

8. Detailed description of some actual analytical system trials using hospital autopsy samples.

1. <u>Investigation of the characteristics for pure samples of the drugs to be screened for</u>: Pure samples of the drugs of interest were acquired from commercial chemical companies, the Bureau of Narcotics and Dangerous Drugs, and the National Institute of Mental Health. The drugs

represented the major classes of drugs used and abused in the United States, including sedatives, tranquilizers, analgesics, stimulants, antihistamines and decongestants, narcotics and miscellaneous, including hallucinogens. These are listed in Table IV under their medical classifications. The chemical name of the drug is given and this is followed in parentheses by the name of the most popular prescription item containing this drug if appropriate.

The drugs were all dissolved in pure methanol at a concentration of 1 mg/ml and stored under deep freeze while not in use. These solutions were used for investigating (a) the thin-layer chromatographic (TLC) and (b) gas chromatographic (GC) characteristics of the drugs.

a. Investigation of the TLC characteristics: To a thin-layer chromatographic plate (20 cm x 20 cm, Silica Gel as on glass, 250 μ thick) drug solutions were spotted on a horizontal line 2 cm from the bottom of the plate. Ten microliters of solution was spotted in each case. Each drug was spotted on at least two different plates. These plates were then developed in glass TLC tanks containing various test solvents. After development of the plates for 10 cm from the spotting line, the plates were removed and dried by an air current. When dry the plates were sprayed with a variety of test visualization reagents and the colors and positions (R_f values) of the drugs noted. The most sensitive and useful solvents and visualization reagents were then used again in duplicate tests to establish reproducibility.

The solvents found superior in these tests were:

Solvent No. 1 Acetone/Chloroform, 1:9
Solvent No. 2 Ethyl Acetate/Methanol/Ammonia, 85:10:5
Solvent No. 3 Ethyl Acetate/Methanol/Ammonia/Benzene, 75:10:2:13
Solvent No. 6 Benzene/Chloroform, 3:7
Solvent No. 11 Benzene

These numbers are not consecutive, but are in accord with the legend to the total developed analytical system referred to later in the script, Figure 3, p. 21.

The visualization reagents found superior in these tests were:

UV - ultraviolet light

$HgSO_4$ - mercuric sulfate solution--suspend 5 g of mercuric oxide in 100 ml water, add 20 ml concentrated sulfuric acid, cool and dilute to 250 ml with water

DPC - Diphenyl carbazone solution--dissolve 5 mg in 50 ml chloroform

$KMnO_4$ - Potassium permanganate solution, 0.1% in water

FBB - Fast Blue B solution--250 mg in 100 ml of 0.1 N hydrochloric acid, followed by a spray with 0.5 N sodium hydroxide

Nin - Ninhydrin--commercially available in aerosol bombs

IOP - Iodoplatinate solution--dissolve 1 g platinum tetrachloride in 100 ml water, mix with 300 ml water containing 10 g potassium iodide. Dilute to 400 ml with water. Dilute 1:1 with hydrochloric acid before use.

Table BI, located in Appendix B "Analytical Development," attached to this report, indicates the two solvents found most suitable for the drugs of interest. The most suitable visualization reagents are also shown. Tables BII and BIII (Appendix B) list the mobilities and color reactions for all the drugs using these solvents and visualization reagents.

b. Investigation of the GC characteristics: Aliquots of standard drug solutions were treated with acid or base to release the free drug and then subjected to GC analysis.

Two columns were developed for these drugs--a 6-ft and a 4-ft column, 2 mm and 4 mm ID, respectively, packed with 3% OV-1 on 100/120 mesh Gas Chrom Q. The carrier gas was nitrogen at a flow rate of 50 ml/min, detector temperature was 260°C, injection port was 240°C. The column temperature was varied. The instrument used in these investigations was a Bendix 2500 Gas Chromatograph. Table BIV (Appendix B) lists the drug, column used, column temperature and retention time for all the drugs of interest. The cannabinoids (marihuana) were excluded from this test since they were analyzed for by TLC only (qualitative). Reproducibility was ascertained for the retention times by duplicate runs. The barbiturates were run either as free barbiturates or as methylated derivatives depending on the retention time desired. Morphine and dilaudid had to be methylated to produce reasonable retention times. Methylation was produced on-column using standard commercial methylation reagents. Acetyl salicyclic acid and salicytic acid were silylated before injection to produce useful retention times.

2. Investigation of extraction systems on body fluid solutions of drugs to be screened for: Two types of extraction systems were considered for this program--liquid extraction with diethyl ether and ion-exchange resin column extraction with XAD-2 resin.* The two methods and their comparison are described below.

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* Available from Brinkman Inst., Inc., or Rhom and Haas, Inc.

a. Liquid extraction: A volume of body fluid was treated as follows:

Urine - Take 20 ml,
Blood - Take 15 ml, dilute 1:4 with water
Bile - Take 10 ml, dilute 1:4 with water

The fluid was then taken to pH 2.2 with hydrochloric acid and shaken with an equal volume of diethyl ether. The phases were allowed to separate and the ether phase removed and allowed to evaporate to a residue. The aqueous phase was taken to pH 9.3 and extracted again with an equal volume of ether. Another extraction at pH 11.0 provided a third residue. These residues were taken up in 100 µl of methanol and subjected to thin-layer chromatography and gas chromatography.

b. Ion exchange resin extraction: The body fluids, diluted as for liquid extraction were placed over a column of XAD-2 resin as shown in Figure 2. The pH of the fluid was adjusted to 9.2 with a buffer solution and the fluid allowed to run through the column. The fluid was then discarded and the column washed with 20 ml water. The drugs were then eluted from the column using 20 ml of 1,2-dichloroethane/ethyl acetate, 4:6. This eluate was evaporated to dryness on a water bath at 60°C after the addition of 1 drop of concentrated hydrochloric acid. The residue was reconstituted in 100 µl methanol and subjected to thin-layer chromatography and gas chromatography.

c. Comparison of extraction methods: In order to compare these extraction systems for usefulness with the TLC and GC analysis methods and to determine sensitivity limits for the total analysis method; body fluids were spiked with standard drug solutions, carried through the extraction process and subjected to TLC and GC.

(1) Urine: Four hundred milliliters of urine collected from Midwest Research Institute personnel was divided into four portions. One portion was used as a blank and the other three were spiked as follows:

Blank - No drugs added

Barbiturates - 200 µg phenobarbital, 200 µg secobarbital

Amphetamines - 400 µg d-amphetamine, 4 µg methamphetamine

Narcotics - 400 µg methadone, 400 µg cocaine, 400 µg dilaudid
200 µg morphine, 200 µg codeine, 200 µg demerol
200 µg quinine, 50 µg nicotine

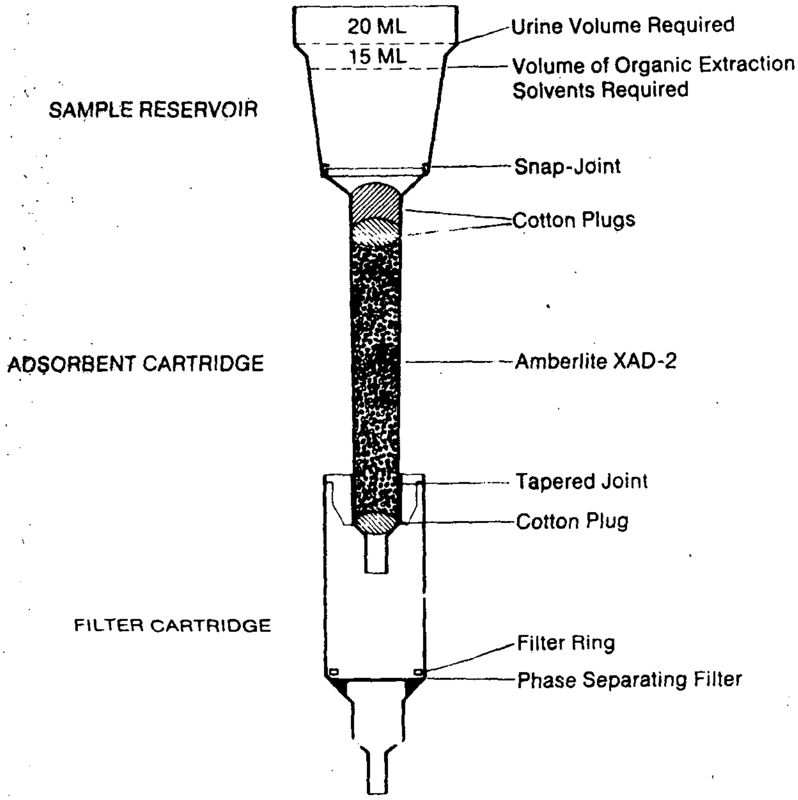


Figure 2 - Extraction Assembly

The above solutions were extracted by both methods, and the extracts subjected to TLC and GC.

The experiments were repeated until reproducible results were obtained using any one batch of body fluid. The results for the ether and resin extraction methods are shown below. In all cases, the resin method gave cleaner extracts than the ether method. The resin method also yielded better extraction of many kinds of drugs, especially morphine. At the level tested, amphetamines and nicotine were detectable only by the resin extract method.

Drugs Spiked Into Urine	Drugs Found in the Extraction Method	
	Ether	Resin
Blank	Negative for all drugs	Negative for all drugs
Barbiturates	Secobarbital Phenobarbital	Secobarbital Phenobarbital
Narcotics	Methadone Cocaine Dilaudid Morphine (weak) Codeine Demerol Quinine	Methadone Cocaine Dilaudid Morphine (very strong) Codeine Demerol Quinine Nicotine
Amphetamines	Negative for both	d-Amphetamine Methamphetamine

(2) Blood: One hundred milliliters of blood (from a local blood bank) was diluted with 400 ml of water and divided into 100 ml portions. The portions were spiked with the same drugs and in the same concentrations as in the case of urine. The blood used in these experiments was treated with heparin or sodium oxalate to prevent coagulation and with sodium fluoride to preserve the blood. Extraction procedures employed were the same as those for urine and were followed by the same TLC and GC procedures as used for urine extracts. In addition, deproteinization of the blood was attempted to research the effect of such a treatment on the extraction processes. Deproteinization detracted much from the efficiencies of both ether and resin extraction schemes. On whole blood and serum the resin extraction method gave remarkably clear extracts which contained more drug than the ether extracts. Amphetamines and nicotine were detectable only when using the resin method. The results are shown below.

Drugs Spiked Into Blood	Drugs Found in the Extraction Method: Ether	Drugs Found in the Extraction Method: Resin
Blank	Negative for all drugs	Negative for all drugs
Barbiturates	Secobarbital Phenobarbital	Secobarbital Phenobarbital
Narcotics	Methadone Cocaine Dilaudid Morphine Codeine Demerol Quinine	Methadone Cocaine Dilaudid Morphine Codeine Demerol Quinine Nicotine
Amphetamines	Negative for both	d-Amphetamine Methamphetamine

These results were also confirmed by GC, yielding extraction efficiencies which were unreproducible and varied from 30-60% with the ion exchange resin.

(3) Bile: A similar experiment using spiked bile (diluted 1:4 with water) yielded results similar to those obtained for blood. Again, extraction efficiencies were on the order of 30-60% using the ion exchange resin.

The ion exchange extraction experiments were then all repeated using all the drugs quoted in Table II (except cannabinoids). All the drugs were detectable with TLC and GC (qualitatively) down to a spiking level of 1 μg/ml for all drugs except salicylic acid and acetylsalicylic acid which could not be detected below 5 μg/ml.

On the basis of these experiments it was concluded that acceptable sensitivity limits were attainable by using ion-exchange resin extraction combined with TLC and GC. Variations in recovery (extraction efficiency) were believed due to reaction and/or destruction of the spiked drugs in the body fluids before extraction, since duplicate extractions from the same spiked body fluid yielded reproducible results.

3. Determination of extraction efficiencies: It was found in previous work that spiking actual body fluids with low levels of drugs (1-2 μg/ml) resulted in unreproducible extraction efficiencies between

TABLE II

DRUGS TO BE INCLUDED IN THE ANALYTICAL SCREEN

Sedatives and Hypnotics

Phenobarbital
Pentobarbital (Nembutal)
Amobarbital (Amytal)
Secobarbital (Seconal)
Butabarbital (Butisol)
Butobarbital (Butethal)
Diphenylhydantoin (Dilantin)
Meprobamate (Miltown)
Glutethimide (Doriden)
Methaqualone (Quaalude)

Tranquilizers

Chlordiazepoxide (Librium)
Diazepam (Valium)
Chlorpromazine (Thorazine)
Promazine
Thioridazine (Mellaril)
Trifluoperazine(Stelazine)

Analgesics

Acetylsalicylic acid (Aspirin)
Salicylic acid
Propoxyphene (Darvon)

Stimulants and Antidepressants

Methylphenidate (Ritalin)
Imipramine (Tofranil)
Amitriptyline (Elavil)
Amphetamine (Dexedrine)
Methamphetamine (Desoxyn)

Antihistamines and Decongestants

Choorpheniramine
Diphenhydramine
Tripecanine
Methapyrilene
Phenylpropanolamine

Narcotics

Nalorphine (Nalline)
Morphine
Codeine
Demerol
Cocaine
Methadone (Dolophine)
Dilandid

Miscellaneous

Dimethyltryptamine (DMT)
Diethyltryptamine (DET)
Lobeline
Mescaline
Methylene dioxyamphetamine (MDA)
Quinine
2,5-dimethoxy-4-methylamphetamine (STP)
Nicotine
Tetrahydrocannabinol (THC)[a]
Cannabinol (CBN)[a]

a/ Ingredients of marihuana.

different samples of the same body fluid (e.g., urine). We came to the conclusion that this was due to reaction of the small amount of drug with the varying ingredients in body fluids. The extraction efficiency was sufficiently large to give the method a useful sensitivity but not reproducible enough for quantitation of drugs in the body fluids.

It was therefore decided to calculate the extraction efficiencies from water and make the assumption that the same extraction efficiency would hold for body fluids. This is a valid assumption since body fluids are mainly water, the ion-exchange resin is capable of extracting 1,000 times the amount of body fluid we actually use, and we are detecting only the free drugs.

Another assumption made was that the extraction efficiency at 10 or 20 μg/ml would be the same as at 1 or 2 μg/ml. To test this, the extraction efficiency of phenobarbital was investigated at levels of 20 μg/ml, 10 μg/ml and 5 μg/ml in water. Ultraviolet spectroscopy of the initial solutions, the water solution after passage through the ion exchange column, and the eluates from the columns, indicated that the extraction efficiency was 75% at all spiking levels. This was also confirmed by gas chromatography. It was found necessary to reconstitute all column eluates in at least 1/2 ml of methanol to avoid loss of drug from the residue vessels. This 1/2 ml of solution was then reduced to 100 μl for submission to TLC and GC.

The extraction efficiencies for other drugs were conducted at levels of 10 μg/ml from water. Experiments were conducted in duplicate to ensure reliability. These extraction efficiencies as shown in Table III are used to quantitate levels of drugs found by GC in the body fluids. Extraction efficiencies have only been calculated for those drugs we have encountered in body fluids of fatally injured drivers in this program so far.

4. <u>Investigation of hydrolysis of specimens</u>: Many drugs, when administered, are not only metabolized to some extent but are also conjugated to glucuronic acid as part of the body's effort to aid excretion. These conjugates or glucuronides will not appear on drug analysis screens since only free drug is assayed by most analytical methods. It is desirable, therefore, to break up the glucuronides to the free drug and glucuronic acid. Hydrolysis will accomplish this, and can be conducted by using acid or enzymes.

Acid hydrolysis is fast and efficient, but it also destroys free drug. The extent of destruction depends on the nature of the drug. Enzyme hydrolysis is slow but gentle. The enzyme usually used, β-glucuronidase, breaks down only the glucuronide conjugates.

TABLE III

EXTRACTION EFFICIENCIES FOR DRUGS USING XAD-2 RESIN

Drug	Extraction Efficiency
Meprobamate	49 ± 1%
Glutethimide	73 ± 8%
Phenobarbital	75 ± 10%
Pentobarbital	66 ± 7%
Amobarbital	73 ± 1%
Trifluoperazine	18 ± 1%
Quinine	81 ± 3%
Chlorpromazine	21 ± 1%
Butobarbital	51 ± 2%
Mescaline	34 ± 2%
Amphetamine	55 ± 9%
Methamphetamine	61 ± 6%

Spiked body fluid experiments were conducted as in the extraction investigation, using ion exchange resin columns followed by TLC of the reconstituted residues. However, hydrolysis was conducted before extraction to determine if any detrimental effects were produced by the hydrolysis conditions. The hydrolysis conditions were:

a. Acid hydrolysis: The spiked fluid was taken to pH 2.0 with hydrochloric acid and the autoclaved at 15 psi for 20 min. After cooling, the fluids were extracted.

b. Enzyme hydrolysis: The spiked fluid was taken to pH 5.2 and incubated at 37°C for 18 hr in the presence of β-glucuronidase. The resultant fluids were filtered and then extracted.

The results indicated that acid hydrolysis destroyed quinine and cocaine and that some barbiturates were lost due to volatility. The enzyme method destroyed no drugs and no volatilization of drugs was evident.

It was concluded that enzyme hydrolysis of urine, blood and bile samples was the most suitable method for the analytical scheme for this program.

5. Development of analytical methods for marihuana: Six human volunteers underwent the following experimental procedure in order to examine the feasibility of detecting marihuana components by washing the oronasal areas and fingers.

Each volunteer was swabbed around the mouth, nose, inside the mouth and on the teeth and gums with a cotton ball dipped in ethanol. Fifty milliliters of ethanol were placed in a beaker for this purpose and the examiner wore rubber gloves, holding the cotton ball with metal tweezers. The cotton balls were dipped in the ethanol, squeezed dry and discarded. The thumb and first two fingers of each hand were dipped into the beaker and shaken for 15 sec. The ethanol was then allowed to evaporate in a hood in preparation for analysis.

Each volunteer was then required to smoke a reefer of marihuana. The marihuana used was a good quality government-furnished variety. The volunteers were left to smoke at their own pace although they were kept under strict observation at all times.

The volunteers were then washed with ethanol in a similar manner as prior to smoking. The ethanol was evaporated in a hood in preparation for analysis.

Blank specimens consisted of 50 ml of ethanol which was evaporated to dryness in preparation for analysis.

Spiked specimens consisted of 10 µg each of THC and cannabinol in 50 ml of ethanol. The solution was evaporated in preparation for analysis.

Analysis of the residues from the ethanol solutions was carried out as follows:

a. Volunteers 1, 2, 3, blank and spiked specimens: The residues were dissolved in 1 ml of a 1:1 mixture of benzene and petroleum ether. The solution was placed on an alumina column and washed with 10 ml of the same benzene:petroleum ether solution. The cannabinoids were then eluted with 5 ml of a 1:1 mixture of benzene:chloroform. The eluate was evaporated to 1/2 ml and spotted on a TLC plate. The plate was developed in benzene and sprayed with Fast Blue B, followed by sodium hydroxide solution (0.5N). A standard solution of THC was applied to each plate before developing the check on the validity of the results.

It was found that in all cases, the washings showed either no cannabinoids present or extremely faint indications of their presence. The standard THC spot showed up very well in all cases. The conclusion is that the cannabinoids were trapped on the column. Further elution did alleviate the problem.

b. Volunteers 4, 5, 6, blank and spiked specimens: The residues were dissolved as much as possible in 1 ml of methanol. Surplus fat was physically removed from the solution. Methanol solution (1/2 ml) was spotted onto TLC plates along with a standard spot of THC. The plates were developed in benzene and sprayed with Fast Blue B followed by 0.5N sodium hydroxide.

In the case of the blank specimen, no detectable traces of cannabinoids were found. Likewise with all three "before smoking" washes--no cannabinoids were found. The standard THC spot gave a red spot at R_f 4.0; the spiked wash gave two spots, red at R_f 4.0 (THC), blue at R_f 4.75 (cannabinol). All three "after smoking" washes gave strong bright spots, red at R_f 4.5 (THC) and blue at R_f 4.75 (cannabinol).

The total results are summarized in Table IV. The conclusion is that the column technique, while removing fat from the samples, also removes much or most of the very fat soluble cannabinoids. Elimination of the column purification step results in a spotting solution from which fat may be physically removed. The slight amount of fat remaining has a small effect on the R_f values of the THC and cannabinol found in user samples, but this does not detract from the method or results. The results indicate that the method is feasible, and it has now been determined that cannabinoids can be detected up to at least 2 hr after smoking by this method.

6. Development of a total analytical system for the drugs to be screened for: Using the data generated in the previous part of this section, a total analytical system for all the drugs of interest was developed. This system, as depicted in Figure 3, is capable of detecting the 46 drugs shown in Table II. Sensitivity levels of better than 1 μg/ml drug in body fluid are obtained for all drugs except salicylic acid and aspirin which have a sensitivity level of 5 μg/ml. Figure 3 is also presented in Appendix B followed by a key and a set of notes. The instructions designed to follow Figure 3 for the TLC screening of drugs in blood, urine and bile are also presented in Appendix B as are the instructions for TLC screening for marihuana from alcohol washings.

Should a positive be qualitatively confirmed by the TLC screen, the residue containing that positive is subjected to GC analysis by injection of 5 μl of the methanolic residue solution into a Bendix 2500 Gas Chromatograph, using the conditions cited.

7. Determination of blood alcohol levels: All blood specimens obtained from fatally injured drivers were assayed for blood alcohol. The method employed for this assay was a gas-chromatographic technique using the "head space" method. A small quantity of blood was placed in a serum bottle with a tight-fitting septum and maintained at a constant temperature of 50°C for at least 1/2 hr. Analyses were performed by injecting several microliters of the head space gas above the blood specimen into

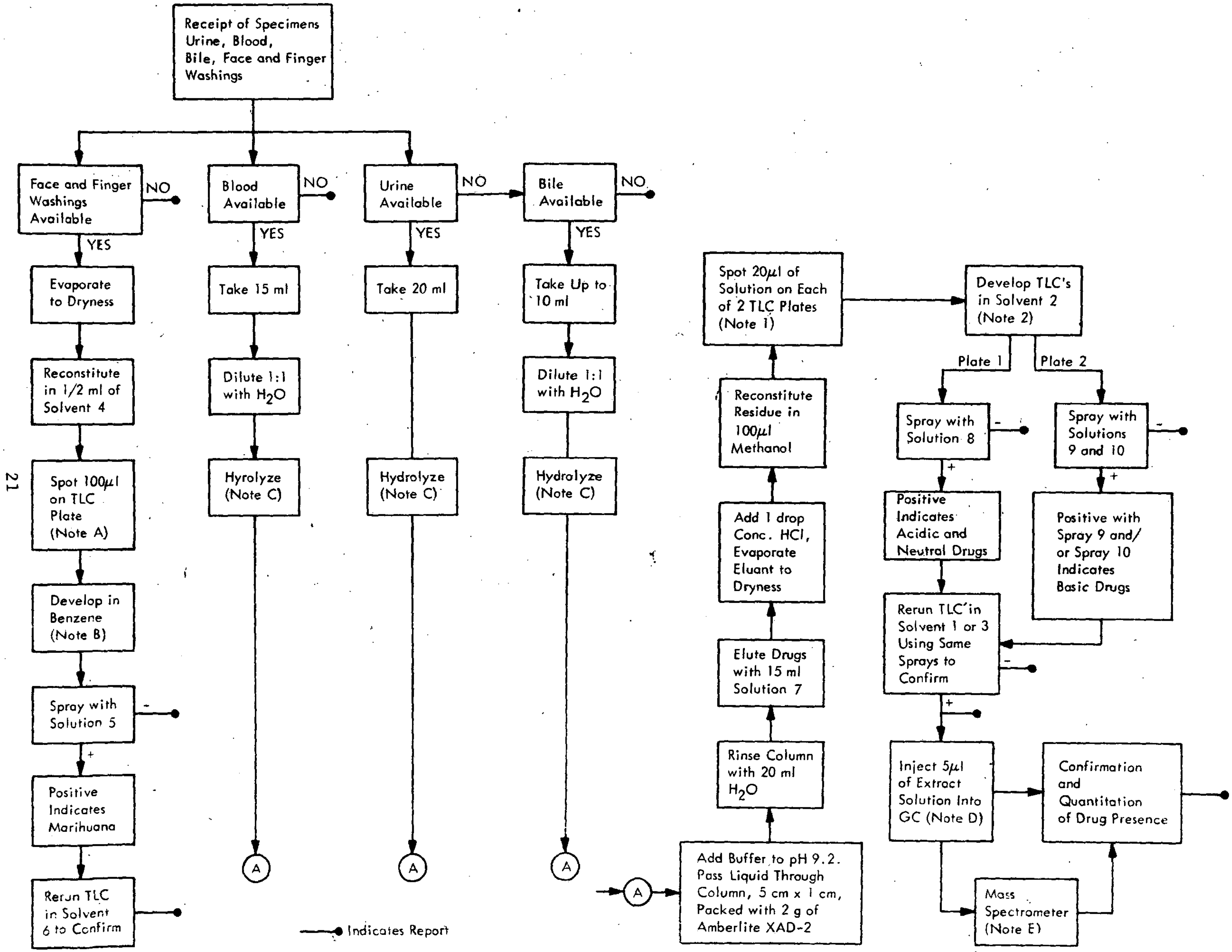
TABLE IV

TLC CHARACTERISTICS OF MARIHUANA ANALYSIS

Specimen	R_f Benzene	Color	Strength
Blank (using column)	-	-	-
Spiked (using column)	-	-	-
User 1 before smoking (using column)	-	-	-
User 1 after smoking (using column)	-	-	-
User 2 before smoking (using column)	-	-	-
User 2 after smoking (using column)	-	-	-
User 3 before smoking (using column)	-	-	-
User 3 after smoking (using column)	-	-	-
Blank (no column)	-	-	-
Spiked (no column)	4.0 4.5	Red Blue	Medium Medium
User 4 before smoking (no column)	-	-	-
User 4 after smoking (no column)	4.5 4.75	Red Blue	Strong Strong
User 5 before smoking (no column)	-	-	-
User 5 after smoking (no column)	4.5 4.75	Red Blue	Strong Strong
User 6 before smoking (no column)	-	-	-
User 6 after smoking (no column)	4.5 4.75	Red Blue	Strong Strong

Figure 3

ANALYSIS OF BODY SPECIMENS FOR DRUGS



a GC with a flame ionization detector. The column was 2 ft by 1/8 in. OD stainless steel packed with 100/120 mesh Porapak Q. The column temperature was held at 110°C and the carrier gas flow was held at 50 cc/min.

These conditions gave good peak shape and separation for ethyl alcohol and acetonitrile, which was employed as an internal standard. A standard curve was prepared over the concentration range 0.050 t0 0.400% by spiking water at these levels and adding a known amount of acetonitrile. These solutions were run on the gas chromatograph and the ratio of the ethyl alcohol to the acetonitrile peak was plotted against percent alcohol. This curve was employed to determine the alcohol concentration in the blood samples by extrapolating the peak height ratios to alcohol concentration.

8. <u>Detailed description of some actual analytical system trials using hospital autopsy samples</u>: Before analysis of fatally injured driver specimens, several autopsy specimens were examined from the local area (Kansas City). In most cases, the drugs administered before death were known. The samples were put through the fully developed screen and confirmation systems. Detailed below are the results of analysis of fluids from four autopsy cases.

Drugs Known Administered	ID No. and Specimen	Analytical Results
Barbiturates	N-71-244 Blood	Phenobarbital 2.75 μg/ml
Demerol and Morphine	N-71-252 Urine	Demerol 72.4 μg/ml Morphine 4.2 μg/ml
	Blood	-
	Bile	-
Demerol	N-71-257 Urine	Demerol 32.1 μg/ml
	Blood	Demerol 6.5 μg/ml
	Bile	Demerol 2.5 μg/ml

Drugs Known Administered	ID No. and Specimen	Analytical Results
Tuinal and MDA*	No ID	
	Urine	Phenobarbital 0.317 μg/ml
		Amphetamine 0.13 μg/ml
	Blood	Phenobarbital 0.47 μg/ml
		Amphetamine trace
	Bile	Phenobarbital 3.65 μg/ml
		Amobarbital 0.217 μg/ml
		Secobarbital 0.155 μg/ml
		Amphetamine trace

Specimen acquisition was not easy, but we believe that these four examples indicate the analytical system developed for this project is most adequate for detecting drugs in fatally injured drivers.

D. Analysis of Specimens from Fatally Injured Drivers

Specimens from 149 fatally injured drivers have been analyzed using the methods developed and described earlier. The procedure adopted for analysis operations was as follows:

1. Specimens are logged in as soon as they arrive. The contents are checked, repackaged if necessary, and frozen until needed for analysis. The ID card is placed in a file and the data from it also entered into a log book and a lab record book.

2. The face and finger washes are analyzed for cannabinoids (marihuana).

3. Five milliliters of blood is removed for alcohol assay.

4. The fluids are hydrolyzed, diluted, extracted and the extracts frozen until needed.

5. The extracts are subjected to a thin-layer chromatographic (TLC) screen.

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* It was indicated that the woman had ingested "Tuinal" tablets known to contain amobarbital and secobarbital. It was also reported that she had taken a street drug which was analyzed by our laboratory and found to contain MDA (methylene dioxy amphetamine) which would be metabolized to amphetamine. Our analytical results agree with these reports. The official laboratory, to which autopsy specimens were also sent, was unable to find amphetamine and could not find any barbiturates at a level consistent with overdose symtoms.

6. Positives from the TLC are run again in a second solvent for qualitative confirmation.

7. Confirmed positives are reconfirmed and quantitated using gas chromatography on the same extract.

8. The extracts are subjected to mass spectrometry if any doubt exists as to the nature of the drug.

9. The results are compiled in a notebook and reports. Quantitation is effected using the GC data in conjunction with the extraction efficiency data.

The results of the analysis of the 149 specimen sets are presented in Section IV of this report.

E. Dissemination of Analytical Information

The analytical data derived from the fatally injured drivers have been compiled in letter form and distributed to the ASAP Regional Directors and others from whom the specimens originated. This service to the coroner and medical examiner has aided in improving cooperation between the parties concerned in this project.

IV. EXPERIMENTAL RESULTS

The experimental results for the 149 fatally injured drivers are listed in Table CI (Appendix C, attached to this report). The sample number corresponds to the number in Table AII--Acquisition of Specimens. Table CI indicates the blood alcohol percent, the gas chromatographic confirmation and drug level in µg/ml, and the alcohol washing for cannabinoids (marihuana) result. The drug level may be converted to mg % by dividing the µg/ml level by ten.

Table CI also indicates the ASAP area or other area from which the specimens came, and whether urine, blood and bile were received in the collection kit.

The analysis and interpretation of these results are presented in the next section.

V. ANALYSIS AND INTERPRETATION OF EXPERIMENTAL RESULTS

In order to fully interpret the analytical results from Table CI, additional pertinent data on the details of the crashes is necessary. To fulfill this need, Crash Data Information Forms as shown in Appendix A were dispatched to each area submitting specimens. The forms were dispatched in duplicate for each specimen submitted.

As of August 31, 1972, we have received only a few Crash Data Information Forms back, and we feel more time and communication are needed in order to get enough forms back to make it possible to use them in an analysis of the analytical results.

The number of fatally injured drivers fully analyzed stands at 149. This is not a statistically significant number for full interpretation of results. However, the following statistics have been compiled on the results.

From the 149 fatally injured drivers, 145 blood samples were collected. Of these 145 blood samples:

71 evidenced no alcohol

74 evidenced alcohol

- of which

16 evidences alcohol $< 0.1\%$

10 evidenced alcohol $\geq 0.1 \leq 0.15\%$

48 evidenced alcohol $\geq 0.15\%$

Percentagewise this indicates:

49% evidenced no alcohol

51% evidenced alcohol

11% evidenced alcohol $< 0.1\%$

7% evidenced alcohol $\geq 0.1 \leq 0.15\%$

33% evidenced alcohol $\geq 0.15\%$

Out of the 71 drivers evidencing no alcohol - 16 evidenced drugs

Out of the 74 drivers evidencing alcohol - 20 evidenced drugs

Out of the 16 drivers evidencing $< 0.1\%$ alcohol - 5 evidenced drugs

Out of the 10 drivers evidencing $\geq 0.1 > 0.15\%$ alcohol - 2 evidenced drugs

Out of the 48 drivers evidencing $\geq 0.15\%$ alcohol - 13 evidenced drugs

Table IV indicates the drug analysis results in condensed form.

Out of the total of 149 drivers, 24% evidenced drugs. Eleven percent evidenced drugs and no alcohol, 13% evidenced drugs and alcohol. Of the 36 drivers with positive drug analyses, the following fluids were available--percentage of fluids having positives is also given.

	Number of Specimens	Number of Specimens Showing Positives	% Showing Positives
Urine	32	24	75.0%
Blood	35	4	11.4%
Bile	31	18	58.1%

Of the 22 drivers with positives greater than trace, the following fluids were available - the percentage of fluids showing positives is also given.

	Number of Specimens	Number of Specimens Showing Positives	% Showing Positives
Urine	20	11	55.0%
Blood	22	3	13.6%
Bile	18	9	50.0%

TABLE IV

COMPILATION OF RESULTS ON DRIVERS INDICATING POSITIVE FOR DRUGS OTHER THAN ALCOHOL

	Fluids Available				Conc.	Drug-Containing Fluid		
Driver ID	Urine	Blood	Bile	Drugs Found	(µg/ml)	Urine	Blood	Bile
				Drivers with 0.0% Blood Alcohol				
6	X	X	X	Amphetamine	0.19	X		
12	X	X	X	Barbiturate	1.66	X		
22	X	X	X	Phenobarbital	tr.*, 1.11	X		X
24	X	0	X	Methamphetamine	tr.			X
31	X	X	0	Phenobarbital	tr., 3.50	X	X	
37	X	X	X	Amphetamine	1.87	X		
75	X	X	X	Amphetamine	tr.	X		
				Methamphetamine	tr.	X		
96	X	X	X	Phenobarbital	tr.	X		
98	X	X	X	Amphetamine	tr.	X		
102	X	X	X	Chlorpromazine	tr.	X		
				Amobarbital	6.16			X
117	X	X	0	Quinine	13.41	X		
124	X	X	0	Chlorpromazine	11.43	X		
128	X	X	X	Pentobarbital	0.33	X		
129	X	X	0	Mescaline	tr.	X		
148	0	X	X	Methamphetamine	1.07			X
149	0	X	X	Butobarbital	tr.			X
				Drivers with > 0.0 < 0.1% Blood Alcohol				
4	X	X	X	Phenobarbital	1.17	X		
34	X	X	X	Glutethimide	0.49		X	
84	X	X	X	Methamphetamine	tr.	X		
85	X	X	X	Methamphetamine	tr.	X		
130	X	X	X	Mescaline	tr.	X		
				Meprobamate	12.24			X
				Chlorpromazine	tr.			X
				Drivers with ≥ 0.1 < 0.15% Blood Alcohol				
35	X	X	X	Mescaline	0.57	X		
127	X	X	X	Amphetamine	tr.	X		
				Butobarbital	tr.			X

TABLE IV (Concluded)

Driver ID	Fluids Available: Urine	Blood	Bile	Drugs Found	Conc. (µg/ml)	Drug-Containing Fluid: Urine	Blood	Bile
				Drivers with ≥ 0.15% Blood				
54	X	X	X	Phenobarbital	2.01, 3.64, tr.*	X	X	X
67	X	X	X	Phenobarbital	4.93, tr.,	X	X	X
72	X	X	0	Amphetamine	0.06	X		
94	X	X	X	Amobarbital	0.34			X
103	X	X	X	Pentobarbital	tr.			X
105	X	X	X	Phenobarbital	tr.			X
				Amobarbital	4.33			X
106	X	X	X	Trifluroperazine	tr.			X
112	0	X	X	Pentobarbital	tr.			X
120	0	X	X	Meprobamate	1.22			X
122	X	X	X	Mescaline	tr.	X		
				Methamphetamine	0.36			X
125	X	X	X	Mescaline	tr.	X		
137	X	X	X	Pentobarbital	tr.			X

* tr. indicates trace quantities (< 0.05 µg/ml).

VI. CONCLUSIONS AND RECOMMENDATIONS

The number of drivers analyzed so far makes it difficult to reach any statistically significant conclusions. However, present data indicate that the incidence of drugs in fatally injured drivers is 24%. However, drunken drivers (blood alcohol ≥ 0.1%) who evidenced drugs account for 10% of the total, so 14% of the fatally injured drivers had ingested drugs but had no alcohol or alcohol levels of less than 0.1%; 11% of the drivers had ingested drugs but no alcohol.

The most frequent drugs found were the barbiturates and amphetamines. Eighteen cases of barbiturates were analyzed and 10 cases of amphetamines in the 149 drivers. Other drugs found included chlorpromazine, glutethimide, mescaline, meprobamate, trifluoperazine and quinine.

Urine samples tended to indicate more drugs than bile samples (75.0% versus 58.1%). Blood samples only indicated positives in 11.4% of the cases.

On the basis of the present results, we recommend that the present project be continued to obtain a statistically significant number of fatally injured driver drug analyses. Moreover, the same screen for 44 drugs should be continued until the incidence of the various classes of drugs can be more accurately ascertained. The face and finger washings for marihuana analysis were 100% negative, and we think this may be due to decomposition of evidence during shipping. If the project is continued, we recommend investigation of possible ways to remedy this situation. We also recommend that blood alcohol analyses be continued as in the study presented in this report.

APPENDIX A

ACQUISITION OF SPECIMENS

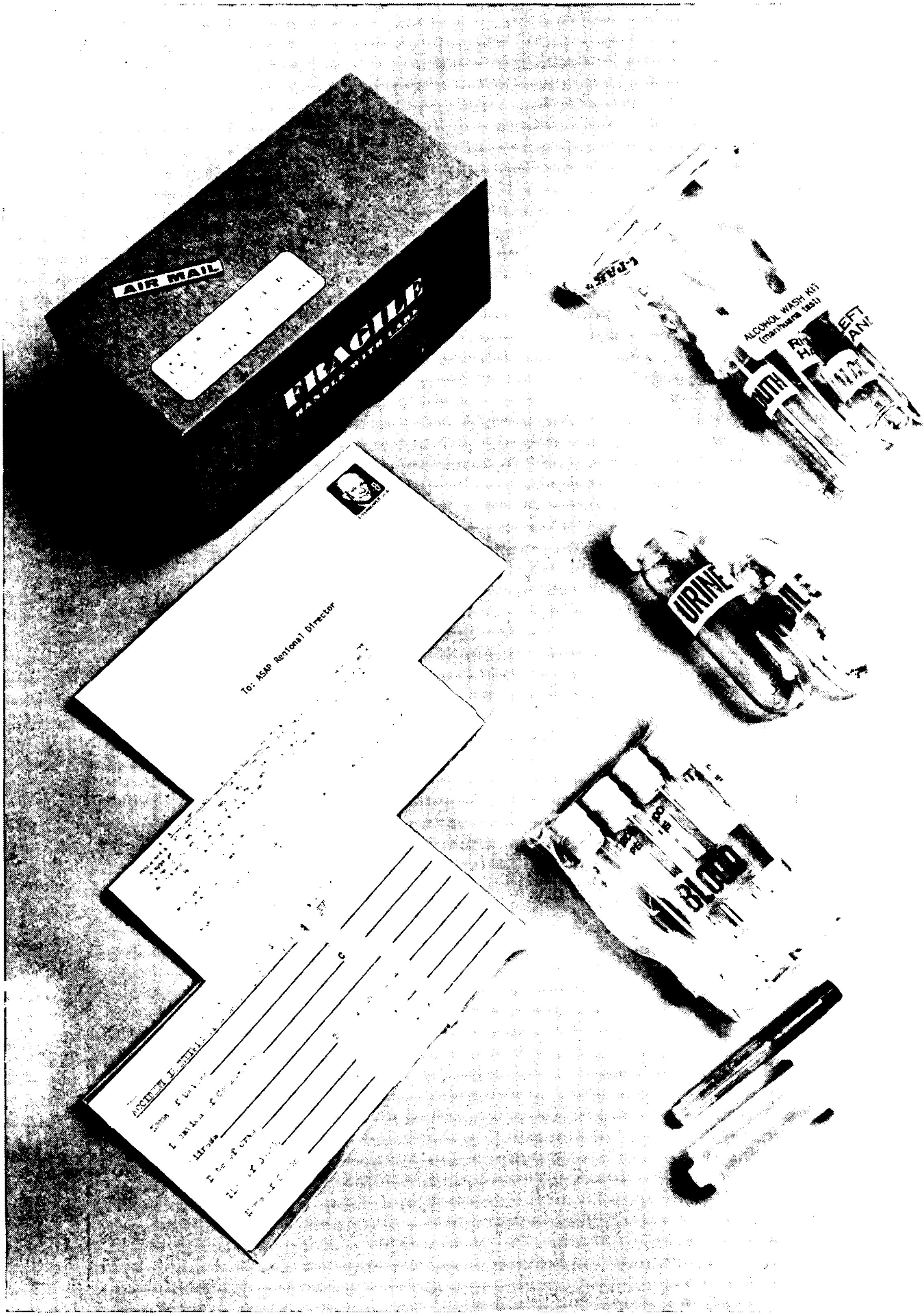

AIR MAIL
FRAGILE
To: ASAP Regional Director
ALCOHOL WASH KIT
URINE
BLOOD

Figure A-1

NHTSA Project DOT-HS-119-1-173, MRI Project No. 3540-C

SPECIMEN COLLECTION FROM FATALLY INJURED DRIVERS

Requirements

The following specimens from fatally injured drivers who are dead on or before arrival at the hospital: (1) blood; (2) urine and/or bile; and (3) alcohol washings of the fingers and face. Please fill out the ID Cards in duplicate. Return one to MRI with the specimens, the other to the ASAP Regional Director in the enclosed envelope.

Instructions for Use of Kit

1. Blood collection: The kit contains a plastic bag with three vacutainer tubes (gray top). A "monoject" double needle (in pink plastic case) and a plastic vacutainer tube and needle holder are also provided.

To collect blood, screw needle into end of tube-and-needle-holder and remove plastic sheath to expose needle. Place a vacutainer tube (gray end first) into the tube holder and contact the gray end with the end of the inner needle. Do not puncture the gray seal at this point. Holding the tube-and-needle-holder with tube inserted, insert the outer needle into blood vessel--be careful not to push on the tube or else the seal will be broken prematurely. When blood vessel is punctured, slowly push the gray ended tube over the inner needle and puncture the gray seal. The vacuum in the tube will draw in approximately 15 ml blood. Remove the gray ended tube of blood and, keeping the needle in the blood vessel, push another empty gray ended tube over the inner needle. Repeat this to produce three vacutainer tubes of blood. Discard the needle, place the three tubes of blood in the plastic bag and secure as when received.

2. Urine collection: The kit contains a plastic screw cap bottle with yellow label "urine." Place as much urine in the bottle as possible, screw the cap back on firmly. No preservative is necessary.

3. Bile collection: The kit contains a plastic screw cap bottle with a green label "bile." This bottle contains preservative which should be kept in the bottle. Place as much bile as possible in the bottle, screw the cap back on firmly and shake to dissolve the preservative.

4. Alcohol washings of the fingers and face: The kit contains a plastic bag with three swabs and a vial of ethyl alcohol. The swabs are marked left hand, right hand, and mouth. Remove the appropriate swab from the swab tube, dip in ethyl alcohol and swab the appropriate area. For the two hands, swab the thumb and first two fingers. For the mouth, swab the area around the lips and the end of the nose. Place the moist swabs back in their respective tubes and place in the plastic bag along with the alcohol bottle.

5. Complete the Identification Card in duplicate. Place one copy in stamped-addressed envelope to the ASAP personnel and mail. Place the other copy in the plastic bag and place back in the kit box.

6. Place all the specimens and ID Card in the kit box, seal with tape along the bottom edge and mail to Midwest Research Institute as soon as possible.

Figure A-2

Instruction Sheet Included in the Specimen Collection Kit

NHTSA Project No. DoT-HS-119-1-173, MRI Project No. 3540-C
ACCIDENT IDENTIFICATION CARD - FATALLY INJURED DRIVER

Name of Driver____

Location of Crash-State__________________County_________

Address____

Date of Crash___________Time of Crash__________________

Time of Death___________Time of Sample_________________

Name of Coroner__________Accident I.D. No._______________

Figure A-3

Identification Card Enclosed in Duplicate in Each Specimen Collection Kit

TABLE A-I

KIT DISPOSITION AS OF AUGUST 31, 1972

ASAP Area	Kits Sent	Kits Returned
Arizona	2	-
Arkansas	22	8
Colorado	50	-
Florida	2	-
Georgia	2	-
Indiana	8	-
Kansas	2	-
Louisiana	2	-
Maine	62	1
Maryland	22	16
Massachusetts	2	-
Michigan	21	8
Minnesota	52	10
Missouri	2	-
Nebraska	14	-
New Hampshire	2	-
New Mexico	50	-
New York	22	1
North Carolina	2	-
Ohio	2	-
Oklahoma	37	7
Oregon	44	33
South Carolina	2	-
South Dakota	1	-
Texas	2	-
Vermont	48	21
Virginia	2	-
Washington	52	29
Wisconsin	26	1
Sacramento, California*	32	20
St. Louis, Missouri*	--	-
San Franciso, California*	20	-
San Diego, California*	20	14
Santa Ana, California*	20	2
Oakland, California*	55	14
San Jose, California*	20	2

* Not ASAP areas.

TABLE A-I (Concluded)

ASAP Area	Kits Sent	Kits Returned
Los Angeles, California*	20	-
San Mateo, California*	20	1
Fort Thomas, Kentucky*	12	-
Everett, Washington*	10	3
Akron, Ohio*	25	-
Martinez, California*	12	-
Atlanta, Georgia*	50	-
San Bernardino, California*	6	-
DoT, Washington, D.C.	50	-
TOTAL	929	191

* Not ASAP areas.

TABLE A-II

SPECIMEN KITS RECEIVED UP TO AUGUST 31, 1972

No.	Date Received	Location of Crash	Specimens Received: Urine	Blood	Bile	Alcohol Washings
1	12/6/71	Washington	X	X	X	X
2	12/8/71	Oregon	X	X	X	X
3	12/15/71	Washington	X	X	X	X
4	12/15/71	Oregon	X	X	X	X
5	12/16/71	Oregon	X	X	X	X
6	12/27/71	Washington	X	X	X	X
7	12/30/71	Oregon	X	X	X	X
8	12/30/71	Washington	X	X	X	X
9	1/5/72	Washington	X	X	X	X
10	1/5/72	Washington	X	X	X	X
11	1/7/72	Washington	X	X	X	X
12	1/10/72	Oregon	X	X	X	X
13	1/13/72	Washington	0	X	X	X
14	1/17/72	Vermont	X	X	X	X
15	1/18/72	Oregon	X	X	X	X
16	1/19/72	Washington	0	X	X	X
17	1/20/72	Minnesota	X	X	X	X
18	1/26/72	Washington	X	X	X	X
19	1/28/72	Vermont	0	X	X	X
20	1/28/72	Vermont	0	X	0	X
21	2/1/72	Vermont	0	X	X	X
22	2/7/72	Washington	X	X	X	X
23	2/16/72	Maryland	X	X	X	X
24	2/16/72	Washington	X	0	X	X
25	2/18/72	Minnesota	X	X	0	X
26	2/29/72	Maryland	X	0	X	X
27	3/3/72	Maryland	X	X	X	X
28	3/7/72	Washington	X	X	X	X
29	3/8/72	Oregon	X	X	0	X
30	3/9/72	Washington	X	X	0	X
31	3/13/72	Sacramento, California	X	X	0	X
32	3/14/72	Oregon	X	X	X	X
33	3/15/72	Sacramento, California	X	X	X	X
34	3/15/72	Wisconsin	X	X	X	X
35	3/24/72	Oregon	X	X	X	X
36	3/24/72	Arkansas	X	X	0	X
37	3/28/72	Maryland	X	X	X	X

TABLE A-II (Continued)

No.	Date Received	Location of Crash	Specimens Received: Urine	Blood	Bile	Alcohol Washings
38	3/28/72	Arkansas	X	X	0	X
39	3/28/72	Sacramento, California	X	X	0	X
40	4/4/72	Oregon	X	X	X	X
41	4/4/72	Sacramento, California	X	X	X	X
42	4/5/72	Oklahoma	X	X	0	X
43	4/7/72	Michigan	X	X	X	X
44	4/7/72	Washington	X	X	X	X
45	4/7/72	Washington	X	X	X	X
46	4/11/72	Vermont	X	X	X	X
47	4/11/72	Sacramento, California	X	X	X	X
48	4/12/72	Washington	X	X	X	X
49	4/12/72	Washington	X	X	X	X
50	4/12/72	Washington	X	X	X	X
51	4/13/72	Oklahoma	X	X	0	X
52	4/17/72	Oregon	0	X	X	X
53	4/19/72	Oregon	X	X	X	X
54	4/19/72	Oregon	X	X	X	X
55	4/20/72	Sacramento, California	X	X	X	X
56	4/21/72	Maryland	X	X	X	X
57	4/21/72	Michigan	X	X	X	X
58	4/24/72	Sacramento, California	X	X	0	X
59	4/25/72	Michigan	0	X	X	X
60	4/25/72	Oregon	X	X	X	X
61	4/25/72	Washington	X	X	X	X
62	4/26/72	Sacramento, California	X	X	X	X
63	5/1/72	Maryland	0	X	X	X
64	5/1/72	Oregon	0	X	X	X
65	5/2/72	Minnesota	X	X	X	X
66	5/2/72	Vermont	X	X	X	X
67	5/3/72	Washington	X	X	X	X
68	5/3/72	Washington	0	X	X	X
69	5/5/72	Sacramento, California	X	X	X	X
70	5/8/72	Minnesota	0	X	X	X
71	5/9/72	Oregon	X	X	X	X
72	5/9/72	Minnesota	X	X	0	X
73	5/10/72	Arkansas	X	X	0	X

TABLE A-II (Continued)

No.	Date Received	Location of Crash	Specimens Received: Urine	Blood	Bile	Alcohol Washings
74	5/10/72	Arkansas	X	X	X	X
75	5/16/72	Washington	X	X	X	X
76	5/16/72	Oregon	X	X	X	X
77	5/17/72	Sacramento, California	X	X	X	X
78	5/19/72	Vermont	X	X	X	X
79	5/22/72	Arkansas	O	X	X	X
80	5/22/72	Maryland	X	X	O	X
81	5/23/72	Washington	X	X	X	X
82	5/24/72	Vermont	X	X	O	X
83	5/24/72	Vermont	X	X	X	X
84	5/24/72	Vermont	X	X	X	X
85	5/25/72	Sacramento, California	X	X	X	X
86	5/30/72	Vermont	X	X	X	X
87	5/30/72	Maryland	X	X	X	X
88	6/1/72	Sacramento, California	X	X	X	X
89	6/1/72	Sacramento, California	X	X	X	X
90	6/02/72	Maryland	X	X	X	X
91	6/5/72	Maryland	X	X	O	X
92	6/5/72	Vermont	X	X	X	X
93	6/5/72	Michigan	X	X	O	X
94	6/5/72	Vermont	X	X	X	X
95	6/6/72	Oregon	O	X	O	X
96	6/7/72	Minnesota	X	X	X	X
97	6/9/72	Oklahoma	O	X	O	X
98	6/12/72	Maryland	X	X	X	X
99	6/12/72	Minnesota	X	X	X	X
100	6/14/72	Oregon	X	X	X	X
101	6/14/72	Sacramento, California	O	X	X	X
102	6/15/72	Arkansas	X	X	X	X
103	6/19/72	New York	X	X	X	X
104	6/19/72	Arkansas	X	X	X	X
105	6/21/72	Oregon	X	X	X	X
106	6/21/72	Oregon	X	X	X	X
107	6/21/72	San Diego, California	X	X	X	X
108	6/25/72	Sacramento, California	O	X	O	X
109	6/26/72	Maine	O	X	O	X
110	6/26/72	Vermont	X	X	X	X

TABLE A-II (Continued)

No.	Date Received	Location of Crash	Specimens Received			
			Urine	Blood	Bile	Alcohol Washings
111	6/26/72	Arkansas	X	X	O	X
112	6/27/72	Michigan	O	X	X	X
113	6/27/72	San Diego, California	X	X	X	X
114	6/27/72	Washington	O	X	X	X
115	6/27/72	Santa Ana, California	X	X	X	X
116	6/27/72	Washington	O	X	X	X
117	6/27/72	Washington	X	X	O	X
118	6/27/72	Santa Ana, California	X	X	O	X
119	6/28/72	Michigan	X	X	X	X
120	6/30/72	San Diego, California	O	X	X	X
121	7/3/72	San Mateo, California	O	X	O	X
122	7/3/72	Vermont	X	X	X	X
123	7/5/72	Maryland	O	X	X	X
124	7/6/72	Minnesota	X	X	O	X
125	7/7/72	Oregon	X	X	X	X
126	7/10/72	Vermont	X	X	X	X
127	7/10/72	San Diego, California	X	X	X	X
128	7/10/72	Oakland, California	X	X	X	X
129	7/10/72	Oregon	X	X	O	X
130	7/10/72	Oregon	X	X	X	X
131	7/11/72	Oklahoma	X	X	O	X
132	7/12/72	Oklahoma	X	X	X	X
133	7/12/72	San Diego, California	X	X	X	X
134	7/13/72	Oakland, California	X	X	X	X
135	7/17/72	Oakland, California	O	X	O	X
136	7/17/72	Oakland, California	X	X	X	X
137	7/19/72	Washington	X	X	X	X
138	7/19/72	Oakland, California	X	X	O	X
139	7/19/72	Oregon	O	X	X	X
140	7/20/72	Michigan	O	O	X	X

TABLE A-II (Continued)

No.	Date Received	Location of Crash	Specimens Received: Urine	Blood	Bile	Alcohol Washings
141	7/20/72	San Diego, California	X	X	X	X
142	7/21/72	Vermont	0	X	0	X
143	7/21/72	Vermont	X	X	X	X
144	7/21/72	Maryland	0	X	X	X
145	7/24/72	Oregon	X	X	X	X
146	7/24/72	Oregon	X	X	X	X
147	7/25/72	Oregon	X	X	X	X
148	7/25/72	San Diego, California	0	X	X	X
149	7/27/72	Oregon	0	X	X	X
150	7/28/72	Oregon	X	X	X	X
151	7/31/72	Everett, Wash.	X	X	0	X
152	7/31/72	Sacramento, California	X	X	X	X
153	8/1/72	Everett, Wash.	0	X	0	X
154	8/2/72	San Diego, California	X	0	X	X
155	8/2/72	Oakland, California	X	X	X	X
156	8/2/72	Sacramento, California	X	X	X	X
157	8/2/72	Sacramento, California	X	X	X	X
158	8/7/72	Oregon	X	X	0	X
159	8/8/72	Minnesota	X	X	X	X
160	8/9/72	Vermont	X	X	X	X
161	8/9/72	San Jose, California	X	X	0	X
162	8/9/72	Minnesota	X	X	X	X
163	8/10/72	Oakland, California	X	X	X	X
164	8/10/72	Sacramento, California	X	X	X	X
165	8/10/72	Oakland, California	X	X	X	X
166	8/10/72	Vermont	0	X	X	X
167	8/10/72	Oklahoma	X	X	0	X
168	8/14/72	Maryland	X	X	0	X
169	8/11/72	Michigan	0	X	X	X
170	8/15/72	Maryland	X	X	0	X
171	8/16/72	Everett, Wash.	X	X	X	X

TABLE A-II (Concluded)

No.	Date Received	Location of Crash	Specimens Received: Urine	Blood	Bile	Alcohol Washings
172	8/16/72	Oregon	X	X	X	X
173	8/17/72	Oakland, California	X	X	X	X
174	8/17/72	Oakland, California	X	X	X	X
175	8/21/72	San Diego, California	X	X	X	X
176	8/21/72	Oakland, California	X	X	X	X
177	8/22/72	Vermont	X	X	X	X
178	8/22/72	Maryland	O	X	X	X
179	8/24/72	San Diego, California	X	X	X	X
180	8/24/72	Oakland, California	X	X	X	X
181	8/24/72	San Diego, California	X	X	X	X
182	8/24/72	Washington	X	X	X	X
183	8/24/72	Oakland, California	X	X	X	X
184	8/24/72	Sacramento, California	O	X	O	X
185	8/28/72	Oklahoma	X	X	O	X
186	8/28/72	Oakland, California	X	X	X	X
187	8/28/72	San Diego, California	O	X	X	X
188	8/28/72	Oregon	O	X	X	X
189	8/30/72	San Diego, California	X	X	X	X
190	8/30/72	San Jose, California	X	X	X	X
191	8/31/72	San Diego, California	X	X	X	X
Total 191			154	186	152	191
Percent of Total Possible			81%	97%	80%	100%

CRASH DATA INFORMATION FORM

(NHTSA Project # DOT-HS-119-1-173)

Note: All information on this form is for research purposes only and is strictly confidential.

Supplier's Name: ______________________

Supplier's Title: ______________________

(and address) ______________________

____

Sample I. D. No. ____________

Date of Crash: ______________________

Time of Crash: ______________________

Time of Death: ______________________

Time and date sample taken: ______________________

Location of Crash:

State: ______________________

County: ______________________

City or Town: ______________________

Address: ______________________

Accident Type - Location (Check one):

a. Single vehicle - Rural /__/

b. Single vehicle - Urban /__/

c. Multiple vehicle - Rural /__/

d. Multiple vehicle - Urban /__/

How many persons killed in crash? ____________

In this driver's vehicle? ____________

In other vehicle (s)? ____________

How many persons injured seriously? ____________

In this driver's vehicle? ____________

In other vehicle? ____________

Collision Type: (Check where applicable)

a. Pedestrian /__/

b. Non-motor vehicle /__/

c. Fixed objects /__/

d. Run off road /__/

e. Overturn /__/

f. Headon . /__/

g. Angle /__/

h. Rear-end /__/

i. Other (specify) /__/

Light Conditions: (Check one)

a. Dawn /__/

b. Daylight /__/

c. Dusk /__/

d. Darkness /__/

Road Surface (Check one)

a. Dry /__/

b. Wet /__/

c. Snowy or icy /__/

d. Other (specify) /__/

Contributing Circumstances (Select condition(s) that most likely contributed to crash.

a. This driver's condition or behavior /__/

b. Other driver's condition or behavior /__/

c. Environment /__/

d. Vehicle condition /__/

e. Other (specify) /__/

Day of Week (Circle one)

Mon Tues Wed Thurs Fri Sat Sun

Vehicle Type(s) (i.e., passenger car, truck, bus, etc.)

This driver's vehicle ____________________

Other vehicle(s) ____________________

Sex of Victim

Male /__/

Female /__/

Age of Victim ______________

** Please submit brief paragraph describing the crash with emphasis on the role of this victim and his vehicle.

APPENDIX B

ANALYTICAL DEVELOPMENT

TABLE B-I

SOLVENTS AND LOCATION REAGENTS FOR TLC OF DRUGS

Drug	Solvents	Location Reagents
Phenobarbital sodium	1 and 2	uv, $HgSO_4$, DPC, $KMnO_4$
Pentobarbital sodium (Nembutal)	1 and 2	uv, $HgSO_4$, DPC, $KMnO_4$
Amobarbital sodium (Amytal)	1 and 2	uv, $HgSO_4$, DPC, $KMnO_4$
Secobarbital sodium (Seconal)	1 and 2	uv, $HgSO_4$, DPC, $KMnO_4$
Butabarbital sodium (Butisol)	1 and 2	uv, $HgSO_4$, DPC, $KMnO_4$
Butobarbital sodium (Butethal)	1 and 2	uv, $HgSO_4$, DPC, $KMnO_4$
Diphenylhydantoin sodium (Dilantin)	1 and 2	uv, $HgSO_4$, DPC, $KMnO_4$
Meprobamate (Miltown)	1 and 2	uv, $HgSO_4$, DPC, $KMnO_4$
Glutethimide (Doriden)	1 and 2	uv, $HgSO_4$, DPC, $KMnO_4$
Acetylsalicylic acid (Aspirin)	1 and 2	uv, $HgSO_4$, DPC, $KMnO_4$
Salycylic acid	1 and 2	uv, $HgSO_4$, DPC, $KMnO_4$
"Quaalude" (Methaqualone HCl)	2 and 3	uv, Nin, IOP
Chlordiazepoxide HCl (Librium)	2 and 3	uv, Nin, IOP
Diazepam HCl (Valium)	2 and 3	uv, Nin, IOP
Chlorpromazine HCl (Thorazine)	2 and 3	uv, Nin, IOP
Promazine HCl (Sparine)	2 and 3	uv, Nin, IOP
Thioridazine HCl (Mellaril)	2 and 3	uv, Nin, IOP
Trifluoperazine HCl (Stelazine)	2 and 3	uv, Nin, IOP
Propoxyphene HCl (Darvon)	2 and 3	uv, Nin, IOP
Methylphenidate HCl (Ritalin)	2 and 3	uv, Nin, IOP
Imipramine HCl (Tofranil)	2 and 3	uv, Nin, IOP
Amitriptyline HCl (Elavil)	2 and 3	uv, Nin, IOP
Chlorpheniramine	2 and 3	uv, Nin, IOP
Diphenhydramine HCl	2 and 3	uv, Nin, IOP
Tripelennamine HCl	2 and 3	uv, Nin, IOP
Methapyriline HCl	2 and 3	uv, Nin, IOP
Phenylpropanolamine HCl	2 and 3	uv, Nin, IOP
Nalorphine HCl (Nalline)	2 and 3	uv, Nin, IOP
Dimethyltryptamine (DMT)	2 and 3	uv, Nin, IOP
Diethyltryptamine (DET)	2 and 3	uv, Nin, IOP
Lobeline HCl	2 and 3	uv, Nin, IOP
Mescaline	2 and 3	uv, Nin, IOP
Methylenedioxyamphetamine HCl (MDA)	2 and 3	uv, Nin, IOP
Amphetamine (Dexadrine)	2 and 3	uv, Nin, IOP
Methamphetamine HCl (Desoxyn)	2 and 3	uv, Nin, IOP
Morphine sulfate	2 and 3	uv, Nin, IOP
Codeine phosphate	2 and 3	uv, Nin, IOP
Demerol HCl	2 and 3	uv, Nin, IOP
Cocaine HCl	2 and 3	uv, Nin, IOP
Methadone HCl (Dolophine)	2 and 3	uv, Nin, IOP
Dilaudid HCl	2 and 3	uv, Nin, IOP
Quinine sulfate	2 and 3	uv, Nin, IOP
2,5-Dimethoxy-4-methylamphetamine (STP)	2 and 3	uv, Nin, IOP
Nicotine	2 and 3	uv, Nin, IOP
Tetrahydrocannabinol (THC)	6 and 11	FBB
Cannabinol (CBN)	6 and 11	FBB

TABLE B-II

TLC R_f (X 10) VALUES AND LOCATION COLORS FOR ACIDIC AND NEUTRAL DRUGS

Drug	R_{f1}	R_{f2}	$HgSO_4$	DPC	$KMnO_4$
Phenobarbital	2.3	2.8	white	violet	white
Pentobarbital	3.3	6.5	white	violet	--
Amobarbital	3.4	5.9	white	violet	--
Secobarbital	3.7	6.3	white	violet	--
Butabarbital	2.8	5.8	clear	purple	--
Butobarbital	2.5	5.5	clear	violet	--
Diphenylhydantoin	1.3	5.4	white	blue	white
Meprobamate	0.3	7.4	clear	white	white
Glutethimide	6.3	9.5	clear	purple	--
Acetylsalicylic acid	0.0	1.8	clear	--	yellow
Salicylic acid	0.0	1.8	clear	--	yellow

Drug	R_{f6}	R_{f11}	FBB
Tetrahydrocannabinol	7.3	6.2	Red
Cannabinol	7.3	6.5	Purple

TABLE B-III

R_f (X 10) VALUES AND LOCATION COLORS FOR BASIC DRUGS

Drug	R_{f2}	R_{f3}	Ninhydrin	Iodoplatinate
Methaqualone	9.5	9.0	--	red/brown
Chlordiazepoxide	7.0	5.5	--	brown
Diazepam	9.5	4.5	--	brown/red
Chlorpromazine	9.5	6.8	--	red/violet
Promazine	8.8	5.8	--	brown/blue
Thioridazine	9.3	6.8	--	brown/red
Trifluoperazine	8.3	7.5	--	blue/violet
Propoxyphene	9.8	9.0	--	brown
Methylphenidate	9.0	2.0	--	gray
Imipramine	9.0	7.0	--	purple/violet
Amitriptyline	9.4	7.3	--	red/brown
Chlorpheniramine	8.0	4.0	--	brown/blue
Diphenhydramine	9.2	7.0	--	brown
Tripelennamine	10.0	7.5	red/purple	red/brown
Methapyrilene	10.0	7.3	purple	blue/brown
Phenylpropanolamine	6.0	2.0	purple	red
Nalorphine	5.36	3.0	--	blue/purple
Dimethyltryptamine	8.4	4.3	--	purple/violet
Diethyltryptamine	9.4	6.0	--	red/brown
Lobeline	10.0	7.2	orange	red/brown
Mescaline	6.0	1.9	purple	red
Methylenedioxyamphetamine	8.0	3.8	purple/red	red/orange
Amphetamine	8.3	4.1	violet	red
Methamphetamine	7.7	3.1	purple	--
Morphine	4.0	1.4	--	blue
Codeine	6.9	2.6	--	blue/purple
Demerol	9.5	6.7	--	violet/purple
Cocaine	9.8	9.3	--	purple/red
Methadone	10.0	9.0	purple	red/brown
Dilaudid	4.0	1.1	red	purple
Quinine	7.9	3.1	--	gray/purple
2,5-Dimethoxy-4-methyl-amphetamine	8.0	3.4	purple	red/orange
Nicotine	9.4	6.6	--	blue/gray

TABLE B-IV

GC RETENTION TIMES AND COLUMN CONDITIONS FOR DRUGS

Drug	Column Length (ft)	Column Temp. (°C)	Retention Time (min)
Codeine	4	250	0.55
Diazepam	4	250	0.79
Chlorpromazine	4	250	0.67
Chlordiazepoxide	4	250	0.95
Nalorphine	4	250	0.20
Promazine	4	250	0.43
Thioridazine	4	250	1.18
Quinine	4	250	4.10
Morphine[a/]	6	265	2.44
Dilaudid[a/]	6	265	2.64
Cocaine	6	240	2.25
Methadone	6	240	1.93
Demerol	6	240	0.67
Methaqualone	6	240	0.71
Chlorpheniramine	6	240	1.34
Propoxyphene	6	240	0.59
Imipramine	6	240	2.29
Lobeline	6	240	0.87
Amitriptyline	6	240	2.17
Phenobarbital	6	240	1.54
Pentobarbital	6	210	1.93
Amobarbital	6	210	1.85
Secobarbital	6	210	2.24
Butabarbital	6	210	1.42
Butobarbital	6	210	1.50
Dipehnylhydantoin	6	210	2.56
Methylenedioxyamphetamine	6	210	2.16
Mescaline	6	210	2.16
Tripelennamine	6	200	3.74
Diphenhydramine	6	200	2.56
Methylphenidate	6	200	0.39
Meprobamate	6	200	1.14
Glutethimide	6	200	2.72
Dimethyltryptamine	6	200	2.05
Diethyltryptamine	6	200	3.07
2,5-Dimethoxy-4-methyl-amphetamine	6	200	1.18
Methapyrilene	6	200	3.86
Trifluoperzaine	6	200	0.98
Acetyl salicylic acid[b/]	6	170	2.64
Salicylic acid[b/]	6	170	2.56
Secobarbital[a/]	6	170	5.71
Phenobarbital[a/]	6	170	9.57

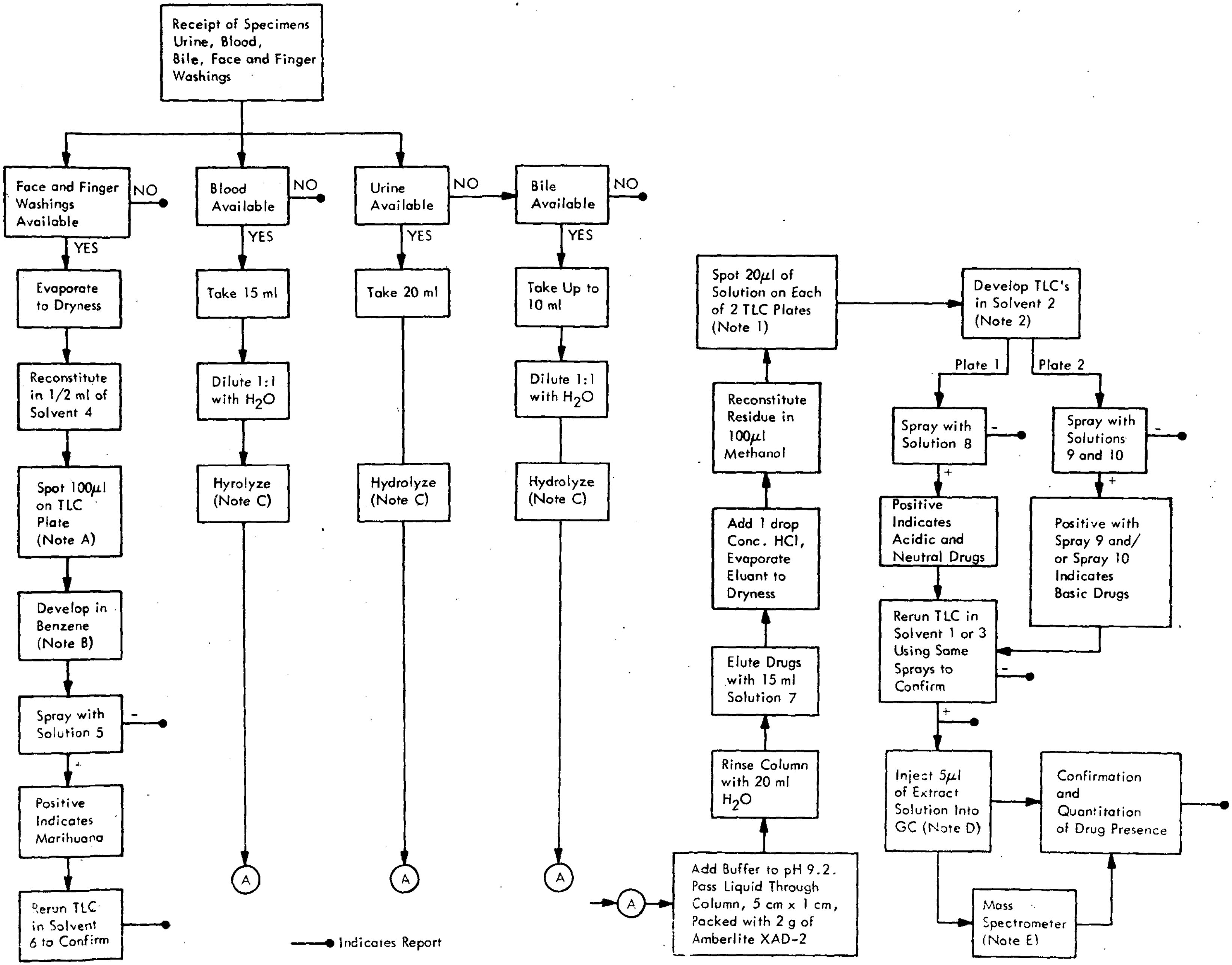
TABLE B-IV (Concluded)

Drug	Column Length (ft)	Column Temp. (°C)	Retention Time (min)
Amobarbital[a]	6	170	4.72
Pentobarbital[a]	6	170	5.12
Nicotine	6	160	1.61
Phenylpropanolamine	6	145	1.06
Methamphetamine	6	145	1.02
Amphetamine	6	145	0.71

a/ Drugs were methylated on-column.

b/ Drugs were silylated.

ANALYSIS OF BODY SPECIMENS FOR DRUGS



KEY TO ANALYTICAL SCHEME

Solvent 1: For acidic and nautral drugs--acetone/chloroform, 1:9

Solvent 2: Ethyl acetate/methanol/ammonia, 85:10:5

Solvent 3: For basic drugs--ethylacetate/methanol/ammonia/benzene, 75:10:2:13

Solvent 4: Benzene/petroleum ether, 1:1

Solution 5: Fast Blue B solution - 250 mg in 100 ml of 0.1N hydrochloric acid. Follow with a spray of 0.5N sodium hydroxide.

Solvent 6: Benzene/chloroform, 3:7

Solution 7: 1,2-Dichloroethane/ethyl acetate, 4:6

Solution 8: Mercuric sulfate solution - suspend 5 g of mercuric oxide in 100 ml water, add 20 ml concentrated sulfuric acid. Cool and dilute to 250 ml with water. Follow with diphenyl carbazone (DPC) solution - dissolve 5 mg DPC in 50 ml chloroform. Follow this with a 0.1N solution of $KMnO_4$ (potassium permanganate).

Solution 9: Ninhydrin - commercially available in aerosol bombs from Brinkmann

Solution 10: Iodoplatinate solution 0 dissolve 1 g platinum tetrahcloride in 100 ml water, mix with 300 ml water containing 10 g potassium iodide. Dilute to 400 ml with water.

Solvent 11: Benzene

NOTES ON ANALYTICAL SCHEME

Note A: Use 20 x 20 cm silica gel G, 250 μ on glass. Spot extracts, along with standards, 1.0 cm from lower edge of plate. Warm the plate slightly when spotting.

Note B: Develop in glass tank with lid. Use solvent to about 0.5 cm depth. Develop the plate 10 cm above spotting line. Remove and dry at room temperature.

Note C: Hydrolyze by adding 3900 Fishman Units of β-Glucuronidase, take to pH 5.2, incubate at 37°C for 18 hr, centrifuge and filter.

Note D: A Bendix 2500 Gas Chromatograph has been employed. Glass columns, 5 ft x 4 mm (ID) with 3% OV-1 on 100/120 mesh Gas Chrom Q. 5 μl of extract solution were injected. Carrier gas is N_2, at a flow of 50 ml/min. Detector temperature 250°C, injection port temperature 240°C. Column temperature between 160° and 265°C depending on drugs being analyzed.

Note E: The mass spectrometer employed in this analytical scheme is a Varian MAT CH-4. This is connected to the gas chromatograph via a Watson-Biemann helium separator. A Varian 8K core laboratory computer and teletype are employed with the GC/MS set-up.

TOXICOLOGICAL SCREEN (RESIN)

BLOOD

a. Take 15 ml blood, or one-half of specimen, whichever is smaller.

b. Spin down, dilute 1:1 with distilled water, add 3900 Fishman Units of β-glucuronidase reagent.

c. Take to pH 5.2.

d. Incubate at 37°C (99°F) for 18 hr, centrifuge, filter.

e. Run through Amberlite XAD-2 column, adding appropriate buffer (pH 9.2).

f. Wash Amberlite column with 20 ml distilled water.

g. Pull dry using aspirator.

h. Elute column with 20 ml of ethyl acetate/cichlorethane (5:4), add 1 drop of HCl.

i. Evaporate eluate to dryness in water bath at 60°C.

j. Reconstitute residue in 0.5 ml methanol and transfer to 1/2 dram vial, evaporate to 100 µl, and label with red tape.

k. Spot 20 µl of residue solution onto each of two 20 x 20 cm TLC plates. Spot standards on the plate, along with any other concurrent analyses.

l. Run the plates for 10 cm in Solvent No. 2, from 2 cm to 12 cm.

m. Dry and spray the plates, one with mercuric sulfate, DPC and $KMnO_4$ for acidic drugs; the other with ninhydrin for amphetamines--followed by iodoplatinate for other basic drugs.

n. Record observations--R_f values and colors--include those of standards.

o. Confirm results by spotting a further 20 µl of residue solution and developing (with standards) in a second solvent (Solvent No. 3 for amphetamines and basic drugs; Solvent 1 for barbiturates).

p. Record all observations. Retain extracts in freezer.

TOXICOLOGICAL SCREEN (RESIN)

URINE

a. Take 20 ml of urine, or one-half of specimen, whichever is the smaller.

b. Add 3900 Fishman Units of β-glucuronidase reagent.

c. Take to pH 5.2.

d. Incubate at 37°C (99°F) for 18 hr, filter.

e. Run through Amberlite XAD-2 column, adding appropriate buffer (pH 9.2).

f. Wash Amberlite column with 20 ml distilled water.

g. Pull dry using aspirator.

h. Elute column with 20 ml of ethyl acetate/dichloroethane (6:4), add 1 drop of conc. HCl.

i. Evaporate eluate to dryness in water bath at 60°C.

j. Reconstitute residue in 0.5 ml methanol and transfer to 1/2 dram vial, evaporate to 100 µl, and label with yellow tape.

k. Spot 20 µl of residue solution onto each of two 20 x 20 cm TLC plates. Spot standards on the plate, along with any other concurrent analyses.

l. Run the plates for 10 cm in Solvent No. 2, from 2 cm to 12 cm.

m. Dry and spray the plates, one with mercuric sulfate, DPC and $KMnO_4$ for acidic drugs; the other with ninhydrin for amphetamines--followed by iodoplatinate for other basic drugs.

n. Record observations--R_f values and colors--including those of standards.

o. Confirm results by spotting a further 20 µl of residue solution and developing (with standards) in a second solvent (Solvent No. 3 for amphetamines and basic drugs; Solvent No. 1 for barbiturates).

p. Record all observations. Retain extracts in freezer.

TOXICOLOGICAL SCREEN (RESIN)

BILE

a. Take 10 ml bile, or one-half of specimen, whichever is the smaller.

b. Spin down, dilute 1:1 with distilled water, add 3900 Fishman Units of β-glucuronidase reagent.

c. Take to pH 5.2.

d. Indubate at 37°C (99°F) for 18 hr, centrifuge, filter.

e. Run through Amberlite XAD-2 column, adding appropriate buffer (pH 9.2).

f. Wash Amberlite column with 20 ml distilled water.

g. Pull dry using aspirator.

h. Elute column with 20 ml of ethyl acetate/dichloroethane (6:4), add 1 drop of conc. HCl.

i. Evaporate eluate to dryness in water bath at 60°C.

j. Reconstitute residue in 0.5 ml methanol and transfer to 1/2 dram vial, evaporate to 100 µl, and label with green tape.

k. Spot 20 µl of residue solution onto each of two 20 x 20 cm TLC plates. Spot standards on the plate, along with any other concurrent analyses.

l. Run the plates for 10 cm in Solvent No. 2, from 2 cm to 12 cm.

m. Dry and spray the plates, one with mercuric sulfate, DPC and $KMnO_4$ for acid drugs; the other with ninhydrin for amphetamines--followed by iodoplatinate for other basic drugs.

n. Record observations--R_f values and colors--including those of standards.

o. Confirm results by spotting a further 20 µl of residue solution and developing (with standards) in a second solvent (Solvent No. 3 for amphetamines and basic drugs; Solvent No. 1 for barbiturates).

p. Record all observations. Retain extracts in freezer.

TOXICOLOGICAL SCREEN FOR CANNABINOIDS

1. Wash the swabs by agitating all three in about 10 ml methanol in the bottom of a 250 ml beaker.

2. Allow the methanol to evaporate in a hood.

3. Reconstitute in minimum amount (1/2 ml or less) of methanol and spot half the residue on a 20 x 20 cm silica gel TLC plate (2 cm from bottom of plate.

4. Spot standards of THC and CBN on the plate along with any other marihuana test specimens. Plate should hold up to 12 tests plus standards.

5. Develop the plate from 2 cm to 12 cm in benzene (Solvent 11).

6. Spray with Fast Blue B, followed by dilute (0.5 N) sodium hydroxide.

7. Note all R_f's and colors.

8. If positives occur, confirm by spotting remaining half of residue and running in benzene/chloroform 3:7 (Solvent No. 6).

9. Record all observations and conclusions.

APPENDIX C

RESULTS

TABLE C-I

ANALYTICAL RESULTS OF SPECIMENS FROM FATALLY INJURED DRIVERS

Sample No.	Area of Origin	Blood Alcohol	Qualitative Screen: Urine	Qualitative Screen: Blood	Qualitative Screen: Bile	Quantitative Confirmation: Urine	Quantitative Confirmation: Blood	Quantitative Confirmation: Bile	Marihuana Analysis
1.	Washington	*	-	-	-	-	-	-	-
2.	Oregon	0.400	-	-	-	-	-	-	-
3.	Oregon	-	-	-	-	-	-	-	-
4.	Oregon	0.038	Pheno	-	-	1.17 ±0.18	-	-	-
5.	Washington	0.250	-	-	-	-	-	-	-
6.	Washington	-	Amphet	-	-	0.19 ±0.03	-	-	-
7.	Oregon	0.200	-	-	-	-	-	-	-
8.	Washington	-	-	-	-	-	-	-	-
9.	Washington	0.185	-	-	-	-	-	-	-
10.	Washington	0.150	-	-	-	-	-	-	-
11.	Washington	0.250	-	-	-	-	-	-	-
12.	Oregon	-	A barbiturate	-	-	~1.66			
13.	Washington	-	*	-	-	*	-	-	-
14.	Vermont	0.425	-	-	-	-	-	-	-
15.	Oregon	-	-	-	-	-	-	-	-
16.	Washington	0.038	*	-	-	*	-	-	-
17.	Minnesota	0.038	-	-	-	-	-	-	-
18.	Washington	0.212	-	-	-	-	-	-	-
19.	Vermont	-	*	-	-	*	-	-	-
20.	Vermont	-	*	-	*	*	-	*	-
21.	Vermont	-	*	-	-	*	-	-	-
22.	Washington	-	Pheno	-	Pheno	(trace)	-	1.11 ±0.17	-
23.	Maryland	-	-	-	-	-	-	-	-
24.	Washington	*	-	*	Meth	-	*	(trace)	-
25.	Minnesota	0.125	-	-	*	-	-	*	-
26.	Maryland	*	-	*	-	-	*	-	-
27.	Maryland	0.236	-	-	-	-	-	-	-
28.	Washington	0.186	-	-	-	-	-	-	-
29.	Oregon	-	-	-	*	-	-	*	-
30.	Washington	-	-	-	*	-	-	*	-

TABLE C-I (Continued)

Sample No.	Area of Origin	Blood Alcohol	Qualitative Screen Urine	Qualitative Screen Blood	Qualitative Screen Bile	Quantitative Confirmation Urine	Quantitative Confirmation Blood	Quantitative Confirmation Bile	Marihuana Analysis
31.	Sacramento, Calif.	-	Pheno	Pheno	*	(trace)	3.50 ±0.53	*	-
32.	Oregon	0.225	-	-	-	-	-	-	-
33.	Sacramento, Calif.	0.062	-	-	-	-	-	-	-
34.	Wisconsin	0.075	-	Gluteth	-	-	0.49 ±0.06	-	-
35.	Oregon	0.100	Mesc	-	-	0.57 ±0.04	-	-	-
36.	Arkansas	-	-	-	*	-	-	*	-
37.	Maryland	-	Amphet	-	-	1.87 ±0.37	-	-	-
38.	Arkansas	0.132	-	-	*	-	-	*	-
39.	Sacramento, Calif.	0.058	-	-	*	-	-	*	-
40.	Oregon	-	-	-	-	-	-	-	-
41.	Sacramento, Calif.	0.088	-	-	-	-	-	-	-
42.	Oklahoma	-	-	-	*	-	-	*	-
43.	Michigan	0.088	-	-	-	-	-	-	-
44.	Washington	-	-	-	-	-	-	-	-
45.	Washington	-	-	-	-	-	-	-	-
46.	Vermont	0.220	-	-	-	-	-	-	-
47.	Sacramento, Calif.	0.375	-	-	-	-	-	-	-
48.	Washington	-	-	-	-	-	-	-	-
49.	Washington	0.105	-	-	-	-	-	-	-
50.	Washington	0.115	-	-	-	-	-	-	-
51.	Oklahoma	-	-	-	*	-	-	*	-
52.	Oregon	-	*	-	-	*	-	-	-
53.	Oregon	0.212	-	-	-	-	-	-	-
54.	Oregon	0.500	Pheno	Pheno	Pheno	2.01 ±0.31	3.64 ±0.56	(trace)	-
55.	Sacramento, Calif.	-	-	-	-	-	-	-	-
56.	Maryland	0.275	-	-	-	-	-	-	-
57.	Michigan	-	-	-	-	-	-	-	-
58.	Sacramento, Calif.	-	-	-	*	-	-	*	-
59.	Michigan	-	*	-	-	*	-	-	-
60.	Oregon	-	-	-	-	-	-	-	-

TABLE C-I (Continued)

Sample No.	Area of Origin	Blood Alcohol	Qualitative Screen Urine	Qualitative Screen Blood	Qualitative Screen Bile	Quantitative Confirmation Urine	Quantitative Confirmation Blood	Quantitative Confirmation Bile	Marihuana Analysis
61.	Washington	0.050	-	-	-	-	-	-	-
62.	Sacramento, Calif.	-	-	-	-	-	-	-	-
63.	Maryland	-	*	-	-	*	-	-	-
64.	Oregon	-	*	-	-	*	-	-	-
65.	Minnesota	0.125	-	-	-	-	-	-	-
66.	Vermont	0.238	-	-	-	-	-	-	-
67.	Washington	0.212	Pheno	Pheno	Pheno	4.93 ±0.76	(trace)	(trace)	-
68.	Washington	0.050	*	-	-	*	-	-	-
69.	Sacramento, Calif.	-	-	-	-	-	-	-	-
70.	Minnesota	-	*	-	-	*	-	-	-
71.	Oregon	-	-	-	-	-	-	-	-
72.	Minnesota	0.175	Amphet	-	*	0.06 ±0.01	-	*	-
73.	Arkansas	-	-	-	*	-	-	*	-
74.	Arkansas	-	-	-	-	-	-	-	-
75.	Washington	-	Amphet	-	-	(trace)	-	-	-
			Meth			(trace)			
76.	Oregon	0.050	-	-	-	-	-	-	-
77.	Sacramento, Calif.	0.375	-	-	-	-	-	-	-
78.	Vermont	-	-	-	-	-	-	-	-
79.	Arkansas	-	*	-	-	*	-	-	-
80.	Maryland	0.212	-	-	*	-	-	*	-
81.	Washington	0.250	-	-	-	-	-	-	-
82.	Vermont	0.151	-	-	*	-	-	*	-
83.	Vermont	0.380	-	-	-	-	-	-	-
84.	Vermont	0.080	Meth	-	-	(trace)	-	-	-
85.	Sacramento, Calif.	0.025	Meth	-	-	(trace)	-	-	-
86.	Vermont	0.320	-	-	-	-	-	-	-
87.	Maryland	0.255	-	-	-	-	-	-	-
88.	Sacramento, Calif.	0.300	-	-	-	-	-	-	-
89.	Sacramento, Calif.	-	-	-	-	-	-	-	-
90.	Maryland	-	-	-	-	-	-	-	-

TABLE C-I (Continued)

Sample No.	Area of Origin	Blood Alcohol	Qualitative Screen Urine	Qualitative Screen Blood	Qualitative Screen Bile	Quantitative Confirmation Urine	Quantitative Confirmation Blood	Quantitative Confirmation Bile	Marihuana Analysis
91.	Maryland	-	-	-	*	-	-	*	-
92.	Vermont	-	-	-	-	-	-	-	-
93.	Michigan	0.455	-	-	-	-	-	-	-
94.	Vermont	0.500	-	-	Amo	-	-	0.34 ±0.01	-
95.	Oregon	-	*	-	*	*	-	*	-
96.	Minnesota	-	Pheno	-	-	(trace)	-	-	-
97.	Oklahoma	-	*	-	*	*	-	*	-
98.	Maryland	-	Amphet	-	-	(trace)	-	-	-
99.	Minnesota	-	-	-	-	-	-	-	-
100.	Oregon	0.130	-	-	-	-	-	-	-
101.	Sacramento, Calif	0.030	*	-	-	*	-	-	-
102.	Arkansas	-	Choroprom	-	Amo	(trace)	-	6.16 ±0.09	-
103.	New York	0.290	-	-	Pento	-	-	(trace)	-
104.	Arkansas	-	-	-	-	-	-	-	-
105.	Oregon	0.325	-	-	Pheno	-	-	(trace	-
					Amo			4.33 ±0.12	
106.	Oregon	0.280	-	-	Trifluo	-	-	2.17 ±0.12	-
107.	San Diego, Calif.	0.220	-	-	Buto	-	-	(trace)	-
108.	Sacramento, Calif.	-	*	-	*	*	-	*	-
109.	Maine	0.510	*	-	*	*	-	*	-
110.	Vermont	0.188	-	-	-	-	-	-	-
111.	Arkansas	-	-	-	*	-	-	*	-
112.	Michigan	0.220	*	-	Pento	*	-	(trace)	-
113.	San Diego, Calif.	-	-	-	-	-	-	-	-
114.	Washington	-	*	-	-	*	-	-	-
115.	Santa Ana, Calif.	0.170	-	-	-	-	-	-	-
116.	Washington	-	*	-	-	*	-	-	-
117.	Washington	-	Quin	-	* *	13.41 ±0.52	-	* *	-
118.	Santa Ana, Calif.	-	-	-	-	-	-	-	-
119.	Michigan	-	-	-	-	-	-	-	-
120.	San Diego, Calif	0.240	*	-	Mepro	*	-	1.22 ±0.03	-

TABLE C-I (Continued)

Sample No.	Area of Origin	Blood Alcohol	Qualitative Screen			Quantitative Confirmation			Marihuana Analysis
			Urine	Blood	Bile	Urine	Blood	Bile	
121.	San Mateo, Calif.	0.310	*	-	*	*	-	*	-
122.	Vermont	0.300	Mesc	-	Meth	(trace)	-	0.36 ±0.04	-
123.	Maryland	0.150	*	-	-	*	-	-	-
124.	Minnesota	-	Chlorprom	-	*	11.43 ±0.57	-	*	-
125.	Oregon	0.150	Mesc	-	-	(trace)	-	-	-
126.	Vermont	0.230	-	-	-	-	-	-	-
127.	San Diego, Calif.	0.130	Amphet	-	Buto	(trace)	-	(trace)	-
128.	Oakland, Calif.	-	Pento	-	-	0.33 ±0.04	-	-	-
129.	Oregon	-	Mesc	-	*	(trace)	-	*	-
130.	Oregon	0.010	Mesc	-	Mepro	(trace)	-	12.24 ±0.26	-
					Choroprom			(trace)	
131.	Oklahoma	-	-	-	*	-	-	*	-
132.	Oklahoma	0.125	-	-	-	-	-	-	-
133.	San Diego, Calif.	0.110	-	-	-	-	-	-	-
134.	Oakland, Calif.	0.080	-	-	-	-	-	-	-
135.	Oakland, Calif.	-	*	-	*	*	-	*	-
136.	Oakland, Calif.	0.200	-	-	-	-	-	-	-
137.	Washington	0.230	-	-	Pento	-	-	(trace)	-
138.	Oakland, Calif.	-	-	-	*	-	-	*	-
139.	Oregon	0.175	*	-	-	*	-	-	-
140.	Michigan	*	*	*	-	*	*	-	-
141.	San Diego, Calif	-	-	-	-	-	-	-	-
142.	Vermont	-	*	-	*	*	-	*	-
143.	Vermont	-	-	-	-	-	-	-	-
144.	Maryland	-	*	-	-	*	-	-	-
145.	Oregon	0.275	-	-	-	-	-	-	-
146.	Oregon	0.300	-	-	-	-	-	-	-
147.	Oregon	-	-	-	-	-	-	-	-
148.	San Diego, Calif.	-	*	-	Meth	*	-	1.07 ±0.11	-
149.	Oregon	-	*	-	Buto	*	-	(trace)	-

* denotes no fluid available.
- denotes negative result
(trace) indicates concentration of less than 0.05 μg/ml.
Mepro denotes Meprobamate.
Gluteth denotes Glutethimide.
Pheno denotes Phenobarbital.
Pento denotes Pentobarbital.
Amo denotes Amobarbital.
Trifluo denotes Trifluoperazine.
Quin denotes Quinine.
Chlorprom denotes Chlorpromazine.
Buto denotes Butobarbital.
Mesc denotes Mescaline.
Amphet denotes Amphetamine.
Meth denotes Methamphetamine.

APPENDIX D

PROJECT PARTICIPANTS

PROJECT PARTICIPANTS

Personnel participating in this project included Dr. E. J. Woodhouse, Senior Chemist, who directed the project, and Mr. R. A. Adams, Associate Chemist, who was responsible for development of analytical/instrumental methods. Assisting on the project were Miss S. Reich, Assistant Chemist, and Miss J. Huerner, Assistant Chemist. Brief Resumes and tasks performed by the above personnel are presented below.

Dr. E. J. Woodhouse, Senior Chemist. Dr. Woodhouse was the Project Leader and was responsible for directing the project, maintaining communiction between DOT, MRI and the sample supply areas, and directing all project personnel in an effort to achieve the aims and goals of the project. Dr. Woodhouse graduated from Nottingham University, England (B.Sc., 1961; Ph.D., 1964). Dr. Woodhouse was a Postdoctoral Fellow at Oregon State University before joining the staff at Midwest Research Institute in 1967. Since then he has directed the Institute's programs involving drug analysis in body fluids. This has involved method development and application to a wide variety of drugs in body fluids for methadone maintenance programs and community treatment centers. Dr. Woodhouse is Project Leader on programs to develop methods for detecting marihuana smokers and LSD users by body fluid analysis. Another study under his direction involves the identification of illicit drug samples from metropolitan areas such as Kansas City, Missouri; Dayton, Ohio; and New York City. Dr. Woodhouse recently completed two projects for the U.S. Army on detection systems for marihuana and heroin users, and a test kit for marihuana plant material.

Mr. R. A. Adams, Associate Chemist. Mr. Adams assumed responsibility on this project for supervising the thin-layer chromatographic screening procedures and for developing and supervising the gas chromatographic and mass spectrometric techniques employed. Mr. Adams graduated from Kansas State College, Emporia (B.A., 1965) and Kansas State University (M.S., 1969). Mr. Adams has had extensive experience with instrumental analysis research including n.m.r., infrared, near infrared spectroscopy. He has drug analysis experience employing mass spectrometry, gas chromatography, GC/MS, and wet chemical techniques. He worked with R. G. Cooks at Kansas State University on the high resolution AEI MS-9, and is currently assisting Dr. Woodhouse in the GC/MS analysis of drug extracts from street drug formulations. Mr. Adams is initiating studies into the feasibility of determination of the origin of opium poppies using gas chromatographic and mass spectrometric techniques.

Mrs. S. Reich, Assistant Chemist. Mrs. Reich was responsible for conducting all extraction and thin-layer chromatographic techniques for the drug screening process. Miss Reich graduated from the University of Kansas, Lawrence, Kansas (B.A., 1960, Microbiology). She has worked 5 years as a research assistant at the Kansas University Medical Center, Kansas City, Kansas. Her experience included analytical and clinical chemistry, morphology and physiology of the placenta, and microbial fermentations.

Miss J. Huerner, Assistant Chemist. Miss Huerner was responsible for conducting blood alcohol assays and the gas chromatographic quantitative analyses for drugs in the body fluids.